INTRODUCTION

Colon cancer is one of the most frequent malignant tumours and the second leading cause of carcinoma-related deaths in developed countries. Advancements in the area of molecular medicine have led to new findings regarding the mechanisms of pathogenesis of this type of carcinoma. These findings have led to new research approaches for potential medications and diagnostic procedures for the prevention and treatment of this type of carcinoma.

Gene p53 is the most frequently mutated gene found in tumours. It is located on chromosome 17, and it encodes the p53 transcription factor. p53 regulates the cell cycle by activating gene transcription, and some p53 gene targets act to arrest the cell cycle in G1, and when necessary, it also initiates programmed cell death (apoptosis); thus, p53 belongs to the group of tumour-suppressing genes. The basic role of a tumour-suppressing gene is to arrest the cell cycle in order to repair errors in the DNA structure. Mutations or the inactivation of tumour-suppressing genes result in uncontrollable cell division and a failure to apoptose. They belong to a group of recessive oncogenes, because they are expressed after both alleles are deactivated.

The wild-type form of p53 takes part in the DNA repair process, controls the cell cycle, cell proliferation and cell differentiation, and under certain conditions, triggers apoptosis by inducing expression of the Bcl-2 gene family. The Bcl-2 family includes both anti-apoptotic (Bcl-2, Bcl-XL) and pro-apoptotic (Bad, Bcl-2-associated X protein (Bax)) proteins. Mutated p53 either loses its tumour-suppressing ability or has it inactivated through the interaction of p53 with other cellular proteins or viral oncoproteins (e.g., HPV E7). Wild-type p53 is expressed in normal tissues at undetectable levels, but the mutated form is expressed in over 50% of all tumours. The method of p53 expression and the level of p53 expression are both significant for monitoring tumour development and prognosis only if it is coexpressed with other tumour markers. However, this is not the case in terminal phases of illness when the success rate of any therapy, as well as the outcome, is almost completely known. Numerous studies have concluded that it is not enough to monitor the expression of only p53 and that other tumour markers must also be monitored, whose behaviours may point to better therapies for malignant diseases.

The objective of this study was to examine the p53 gene expression levels in tumour samples taken from different areas of the resected colon segments from patients with colorectal carcinoma in order to determine if a correlation exists between clinical and morphological parameters and p53 expression levels. The possible prognostic significance of this correlation was also determined.

ABSTRACT

The purpose of this study was to determine the significance of the correlation of clinical-morphological parameters and the expression levels of p53 in colorectal carcinomas. This was a prospective, clinical-experimental study. We believed that there would be a correlation between the expression levels of proto-oncogenes and the pathological stage as well as the degree of histological differentiation of colon cancer. The study researched and evaluated the correlation between p53 expression and the location of colon and rectal carcinomas.

Key words: carcinoma, colon, p53, location

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MATERIALS AND METHODS

This study was a prospective, clinical-experimental study. Postoperative material was obtained from the resected colorectal tumours of 63 patients (male and female) at the Surgical Clinic of the Medical Faculty in Kragujevac. Immunohistochemical analyses were made at the Department of Pathology and Forensic Medicine of KC “Kragujevac” in Kragujevac.

Immunohistochemical-staining procedures included antigen unmasking, blocking endogenous peroxidases, incubating sections with primary anti-serum and staining with the specific antibody, followed by LSAB + HRP, according to the standard protocol 9. DAKO monoclonal mouse anti-p53 (clone DO-7) antibody diluted 1:200 was used to detect p53. The selected threshold value for determining positive or negative expression of p53 was >30% (i.e., a positive readout on >30% of tumour cells was classified as positive for p53 expression). Therefore, the scoring system was based on determining the percentage of immunoreactive tumour cell nuclei as well as determining the intensity of immunoreactive staining (Table 1).

Table 1. Adding the points for the percentage of immunoreactivity and for the intensity gives the complete possible maximal score for the evaluating expression levels.

<table>
<thead>
<tr>
<th>% of immunoreactive nuclei</th>
<th>Intensity of immunoreactive staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>0= &lt; 5% of nuclei staining</td>
<td>0 = no staining of nucleus</td>
</tr>
<tr>
<td>1 = 5% to 30% of stained nuclei</td>
<td>1 = weak intensity of nucleus staining</td>
</tr>
<tr>
<td>2 = 30% to 50% of stained nuclei</td>
<td>2 = mild intensity of nucleus staining</td>
</tr>
<tr>
<td>3 = 50% to 70% of stained nuclei</td>
<td>3 = very intensive staining</td>
</tr>
<tr>
<td>4 = 70% to 90% of stained nuclei</td>
<td></td>
</tr>
<tr>
<td>5 &gt; 90% of stained nuclei</td>
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</table>

Statistical methods

The Mann-Whitney test was used to compare the variable averages of two populations; for two or more populations, the Kruskal-Wallis test was used. The chi-square test and Fischer’s exact test were used to determine the correlation between two descriptive variables.

RESULTS

Most of the primary colorectal carcinomas (CRCs) were located in the sigmoid colon 23/69 (36.5%). In addition, 17/63 (27%) were located in the rectum, 14/63 (22.2%) in the first section of the large intestine (cecum), and the smallest number 9/63; 14.3%) in the proximal and transverse areas of the colon.

Nuclear expression of p53 was present in 33/63 (52.4%) tumours, and the remaining 30/63 (47.6%) had no p53 expression.

Statistical analysis of CRC location and p53 expression

The Kruskal-Wallis test demonstrated that the percentage of cells with nuclear p53 expression varied significantly between areas of the colon (p = 0.000). Nuclear p53 expression in the sigmoid colon was significantly different than that in other areas, but the other areas did not differ significantly from each other (Figure 1). In contrast, there were no signifi-
colon tumours were positive for p53 expression. The tumour location and p53 expression were dependent (p = 0.012) (Figure 2).

There were no significant differences in score between the analysed CRC locations (p = 0.053), although it is indicative (Figure 3).

**DISCUSSION**

Results of the analysis of 63 CRCs obtained in this study demonstrate that most of the primary CRCs were located in the sigmoid colon, 23/63 (36.5%). In addition, 17/63 (27%) were found in the rectum, 14/63 (22.2%) were situated in the cecum, and only 9/63 were in the proximal and transverse areas of the colon. The results obtained in this study are consistent with the results of several published studies 10-14. According to the other studies, most CRCs are located in the sigmoid colon and rectum (75%), followed by the cecum and other colon segments (16%).

We also demonstrated that the nuclear expression of p53 was present in 33 cases (52.4%), and p53 expression was used as a positivity mark in more than 30% of tumour cells. However, the remaining 30 samples (47.6%) had no p53 expression. Our results are consistent with data in the available literature regarding p53 marker expression in CRCs in which p53 expression in all carcinomas was found to be 45%-70%, while the expression of p53 in CRCs was 42%-67% 15,16.

Many studies have researched the numerous mechanisms of tumour progression in different anatomical regions. p53 mutations are more frequent in tumours of distal parts of the colon and rectum, with underlying invasion of blood and lymphatic vessels in these tumours 17,18. This may explain the highlighted aggressiveness and vascular invasion of proximal colon carcinomas with p53 overexpression. The connection and relationship between p53 mutations and other clinical-pathological indicators has not been confirmed. Some authors have demonstrated that the specific p53 mutations and elevated p53 expression are connected with the aggressiveness of distal-region tumours 19,20. According to the available data, the allele deletion of 17p correlates with a higher risk of emergence of outlying metastases, especially with tumours on the colon’s left side 21.

Examination of the difference between the biological behaviour of p53 in tumours and the rectum does not support p53 as an independent prognostic factor for survival in rectal tumours 22 (Figure 4). Immunohistochemically detected p53 has a limited predictive value, especially in proximal colon tumours. However, an increased p53 expression level in CRC was found in distal parties 23.

A high frequency of p53 mutations in left-side tumours (71%) and the rectum (91%) suggest that the molecular mechanisms of tumourigenesis in synchronous left- and right-side tumours are probably different 24. Still, the fact that a Tp53 mutation’s prognostic value regarding a tumour’s biological behaviour is dependent on the tumour’s location remains 25.

According to the literature, CRC genesis begins with a series of mutations through the adenoma-carcinoma sequence: first APC (tumour suppressor), which results in dysplasia, then K-Ras (oncogene), resulting in anaplasia, and lastly p53, which gives a malignant character to the already mutated tissue 26. Cancerogenesis and CRC development include mutations in multiple genes (APC, C-myc, K-Ras, β-catenin, SMAD4). However, the cumulative effects have a bigger role in cancerogenesis than the particular sequence of changes in those genes 26,27. Molecular markers can help determine the risk of developing an invasive carcinoma from premalignant lesions (adenoma). Therefore, gene therapy should target a number of genes involved in cancerogenesis. According to the same data, these tumours have some genetic instability and a loss of their normal karyotype 26-28. Other authors also suggest its value in the progression of the genetic errors 29.

Today, the p53 mutation is the most frequently found mutation in breast, oesophagus and non-small cell lung carcinoma 30,31. p53 is the base of Lane’s functional model of the ‘molecular policeman’. Wild-type p53 is the transcriptional regulator in the G1 phase of the cell cycle 32. When DNA damage occurs, p53 is activated, and through p21, it inhibits cyclin-dependent kinases and the subsequent phosphorylation of proteins needed to enter S phase. The G1 pause allows DNA error repair or the induction of apoptosis and the prevention of mutated cell proliferation 33. p53 mutations result in an accumulation of p53 proteins, leading to greater proliferation, loss of apoptosis, chromosome instability and disruption of differentiation 34. On the other hand, p53-induced cell death can prevent Bcl-2 expression of proto-oncogenes and inhibit apoptosis 35. All previously used literary information states that the majority of CRCs begin through a successive process of sequential genetic and phenotypic changes, i.e., through hyperplasia, adenoma, carcinoma and metastasis.
Some studies have presented CRC cases in which p53 expression was 76%, and in those, the increased expression was linked with a shorter survival period 36-40. Many studies have dealt with the indicators of prognosis in patients with malignant diseases regarding p53 protein accumulation, genetic mutation of the gene encoding it and the loss of heterozygosity of the allele on chromosome 17. These clinical and basic studies suggest that damage to or mutation in p53 leads to excessive genetic amplification of p53 and a loss of cell-cycle and apoptosis control. The level of these changes has represented the degree of unfavourable prognosis for patients, as tumours with p53 hyper-expression have proven to be resistant to radiation and most chemotherapeutics 41. However, some authors have presented a contradictory relationship between p53 expression and survival rate, arguing that the expression has limited value when it comes to estimating the clinical outcome 42,43. Contrary to those, other authors believe it is a clinically significant marker 44. Some studies have shown that p53 expression is an indication of shorter survival 45,46. Vice versa, some regard p53 expression as a better prognostic factor for colon tumours in the Australian population 47. Recent studies point out that only simultaneous expression of K-Ras and p53 can be an indicator of unfavourable prognosis 47.

In thirty-five other studies (twenty-four including immunohistochemical staining), p53 expression was found to be a predictor of an unfavourable outcome (Figure 5). Paradoxically, twenty-four other studies have shown the opposite results 48. Some studies have shown that the expression level does not have a connection with biological behaviour, tumour stage, histological type, location, tumour size, presence of lymph node and venal invasion, perineural invasion, metastases in lymph nodes or outlying metastases (hepatic and peritoneal), all of which disputes the significance of p53 49.

Based on information that the growth of p53-transfected malignant cells is arrested and that mutant p53 gives way to a vaccine therapy, other studies have proposed the possibility of gene therapy 50.

Wild-type p53 is expressed in normal tissues at undetectable levels. However, mutated p53 is expressed in over 50% of all tumours, when it is possible to detect it (commonly through immunohistochemistry). As a multi-marker, whose level and method of expression is monitored in many tumours, it is used to monitor its connection with the development of malignant diseases 5,7.

Today, colon and rectal tumourigenesis is a known process, from adenoma to carcinoma through a series of mutations (as described above). In an attempt to examine the correlation between the three key molecules in cell transformation (APC, K-ras, and p53) in colorectal neoplasm, some studies have monitored the level of expression of these three tumour markers. The results have shown that p53 is expressed in more than 50% of tumours, APC in approximately 50% of tumours and K-ras in less than 30%. Co-expression of p53 and APC was present in 27% of all tumours, and co-expression of all three only in a low percentage (less than 7%). However, the co-expression of all three corresponded with the most aggressive form of disease and thus, an unfavourable prognosis. Based on the gathered results, the authors concluded that K-ras and p53 use two independent genetic mechanisms of cancerogenesis, which when activated together, lead to the development of aggressive forms of the disease 5,7.

Other studies monitored the expression levels of normal and mutated p53 in breast cancer in situ. Identical p53 mutations were found in approximately equal percentages in situ carcinoma and in invasive forms of the disease, suggesting that there is a clonal connection between the in situ carcinoma and invasive disease. Thus, in situ carcinoma formation is the critical moment in the disease development pathway; on the other side, it disproves the modern understanding of the p53 cancerogenesis mechanism, which implies that the accumulation of mutations leads to an invasive form of the disease 51. In these studies, p53 expression was noted in 36% of all tumours, and in 7%, only mutated p53 expression was noted; in 10%, wild-type p53 hyper-expression was observed, while in 19%, mutated p53 was expressed and wild-type p53 was hyper-expressed 1.

In other mutant p53 studies, the effects of recessive and transdominant mutants were examined; these differed in only one amino acid, and this was the consequence of an error on codon 72. However, the structural difference in only one amino acid differentiated the paths of inhibition of wild-type p53 and mutant p53. The recessive mutation directly inhibited wild-type p53 and the transdominant indirectly inhibited it through p73. This is significant, because recessive mutants are formed more frequently in the process of tumourigenesis (73%) 7.

The occurrence of any of the two mutants in heterozygotic form with wild-type p53, through the inhibition of the latter, allows the coexistence of both wild-type and mutated p53 in the tumour, where both exert their effects. This explains the results of studies that have shown hyper-expression of both wild-type p53 and mutated p53 in the cases with the highest tumour p53 expression. In fact, the smallest percentage (some study) of tumours expressed only mutated p53, but those tumours were the most aggressive. Thus, balance exists in wild-type/mutant p53 coexistence: mutant p53 develops its tumourigenic effects, and wild-type controls them, preventing an already severe disease from becoming even more dangerous 8.

The results of the mentioned studies are somewhat contradictory, since it is now known that tumourigenesis occurs only if both p53 alleles mutate or are missing, meaning that while p53 is heterozygote, one normal p53 is preserved. The previously mentioned studies have shown that p53 is the watchdog of cell functions and can drive the cell in two directions: apoptosis or uncontrollable cell division. However, some studies noted wild-type p53 hyper-expression in tumours when mutant p53 was not expressed. In such cases, it is presumed that some other genetic pathway is activated and that it crosses over with wild-type p53’s expression pathway, as is the case with mutated Mdm-2, which now would not be able to inhibit p53 expression 52,53.

The results of these studies lead to the conclusion that it is not enough to monitor only p53 expression for prognosis, but that the expression of other tumour markers whose behaviours may point to some better therapeutic approaches for malignancies must also be monitored 54-57.

A number of recent studies have monitored the correlation of p53, Ki67, HER2 and hormonal receptor expression with clinical-pathological parameters in ductile breast carcinoma. The size of the tumours has been correlated with HER2+, ER+ and Ki67 expression, and necrosis has been correlated with p53 expression. ER-Pr-HER2+p53+ status was the sign of an unfavourable prognosis 8.
CONCLUSION

Other studies have examined the predictive factors of metastases genesis in the breast cancers of patients undergoing a mastectomy during postoperative radiotherapy. These studies monitored HER2, p53 and Bcl-2 expression and demonstrated that HER2 and p53 were independent predictors of breast carcinoma metastasis. Bcl-2 has also been identified in 38% of tumours, and it correlates with a small tumour size, low histological grade and the lack of metastases. p53 was expressed in 19% of tumours and correlated only with ER+ status. HER2 was expressed in 53% of tumours and correlated with a high number of metastases and a high histological grade. These results suggest that HER2 can be referred to as an independent indicator of disease prognosis, but p53 expression can only be used as a prognostic factor when coexpressed with HER2.

Studies of tumour marker expression on tumour cells isolated from the bone marrow of breast cancer patients (early stage) have shown that early metastasis is rare in patients with ER+PrP+ primary tumours, while it is more frequent in HER2+ primary tumours. However, studies have not examined the correlation between early metastasis occurrence and p53-/p53+ primary tumours.

Studies examining the expression of the potential prognostic factors pRB/p130, p107, p27kip1, p53, Mdm-2 and Ki67 in prostate carcinoma have shown that the expression of p53, Mdm-2, Ki67 and low-level expression of p27kip1 correlates with an unfavourable prognosis. It has also been shown that RB mutations (tumour-suppressor) are early events in tumorigenesis and that p53 expression is not a good prognostic factor in the early stage of disease.

Changes in p16, p53, SMAD4 and Ki67 expression in intraductal papillary-mucinous pancreatic tumours are also present in pancreatic adenoma. However, mutations in these molecules were more frequent in pancreatic cancer, which supports the theory that the invasiveness of tumours rises alongside the accumulation of mutations, meaning that the prognosis is more unfavourable. In Barrett’s adenocarcinoma, there is an inverse relationship between the expression of mutated p53 and p21.

The results of the mentioned studies suggest that p53 expression can only be used for disease prognosis when it is coexpressed with other tumour markers. A direct correlation between p53 expression and prognosis is noted in the later stage of the disease, when any therapy is questionable, meaning there is no significance in measuring the level of its expression.

CONCLUSION

The greatest number of colorectal carcinomas is located in the sigmoid flexure. There is a correlation between p53 expression and location, meaning that the location and p53 are dependent. The percentage of p53 nuclear expression in the sigmoid flexure is significantly different from the expression percentages in other locations, while the differences between other locations are not statistically important, but are indicative. On the basis of the previously stated facts, we conclude that p53 does not have prognostic potential, but its value regarding the understanding of oncogenesis is undisputable.

REFERENCES


