

Expression of p53 and Ki-67 as Prognostic Factors for Survival of Men with Colorectal Cancer*

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Abstract. *In patients with colorectal cancer (CRC) several independent prognostic factors are well-supported in the literature, including TNM stage, histological type and grade, and serum levels of carcinoembryonic antigen (CEA). All cancer cells express high levels of tissue proliferation markers, such as Ki-67 and p53, which are currently considered prognostic markers for patients with several types of cancers. We retrospectively studied 31 men (median age 65, range 48-75 years) with confirmed Dukes' B colorectal adenocarcinoma. The following parameters were recorded: age of the patients (years), baseline CEA serum levels (ng/ml), Ki-67 and p53 expression (%), and survival (months). The mean overall survival was 37.3±13.7 months. The mean baseline CEA serum level was 79±7.4 ng/ml, while the percentage positivity for Ki-67 and p53 in cancer tissues was 46.9±19.2 and 48.7±14.2, respectively. There was a significant correlation between Ki-67 and p53 expression ($R=0.82$, $p<0.001$) and an inverse relationship between survival and the expression of both Ki-67 ($R=-0.67$, $p<0.001$) and p53 ($R=-0.64$, $p<0.001$). No significant correlation was found between survival and age ($R=0.22$, $p=0.22$) or CEA ($R=0.08$, $p=0.67$). There was no relationship between CEA and age ($R=0.34$, $p=0.06$), Ki-67 ($R=-0.021$, $p=0.90$) or p53 ($R=0.03$, $p=0.87$). In conclusion, our preliminary results showed that both Ki-67 and p53*

overexpression in CRC are associated with a worse outcome. In this selected group of patients, these prognostic markers were independent of age, and the preoperative CEA serum levels did not have any relationship with survival.

In patients with colorectal cancer (CRC), several independent prognostic factors are well-supported in the literature, including TNM stage, histological type and grade, and serum carcinoembryonic antigen (CEA) levels (1, 2). All cancer cells express high levels of tissue proliferation markers, such as Ki-67 and p53, which are currently considered prognostic markers for patients with several types of cancer (3).

Usually, overexpression of proliferation markers is associated with worse outcome, but may also be implicated in a better response to chemotherapy (4). Han *et al.* (5) showed that in patients with triple-negative breast cancer, high expression of Ki-67, but not of p53, was significantly associated with axillary nodal metastasis. Thus, Ki-67 and p53 protein play a crucial role in the pathogenesis of a large number of malignancies, while their prognostic value in CRC is still debated (6, 7).

The aim of this retrospective study was to assess the relationship between survival and p53 and Ki-67 overexpression in cancer cells in patients with CRC who underwent curative surgery.

Patients and Methods

Charts of a homogeneous group of 31 men (median age 65, range 48-75 years) with confirmed Dukes' B colorectal adenocarcinoma were reviewed. The following parameters were recorded: age of the patients (years), baseline serum CEA level (ng/ml), Ki-67 and p53 expression (%), and survival (months). All patients had undergone radical surgical resection of histopathologically-confirmed primary tumor. The absence of distant metastases was confirmed using total-body ¹⁸F-fluorodeoxyglucose positron-emission tomography (FDG-PET).

Immunostaining of proliferation markers was performed on archival formalin-fixed and paraffin-embedded tissues, using 5- μ m standard sections (7). Monoclonal antibodies were used for both Ki-67 (MIB-1; Novocastra Laboratories, Newcastle upon Tyne, UK) and

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p53 (clone DO-7; Dako, Glostrup, Denmark) detection and a rabbit immunoglobulin fraction was used as a negative control (5). The Ki-67 and p53 protein immunoreactivity and intensity were semi-quantitatively evaluated in at least 500 cells, examined at 40× magnification, and recorded as a percentage of the total number of neoplastic cells present in the same area (8). The staining was obtained using an automated immunostainer, using a catalyzed signal amplification method (9). Serum CEA levels were determined by automated testing based on a two-site enzyme-linked immunosorbent assay (ADVIA Centaur XP Immunoassay System; Siemens, München, Germany), according to the manufacturer's instructions. A cut-off limit of 10 ng/ml was used, as previously reported (10, 11).

All data presented are expressed as the mean±standard deviation (SD). The Ki-67 and p53 expression rates were correlated with months of postoperative survival using Pearson's correlation test. To address the causative relationship, Pearson's correlation coefficient (R) was calculated. A two-sided error level of $p < 0.01$ was considered statistically significant.

Results

The overall mean survival was 37.3 ± 13.7 months. The mean baseline CEA serum level was 79 ± 7.4 ng/ml, while the percentage positivity for Ki-67 and p53 in cancer tissues was 46.9 ± 19.2 and 48.7 ± 14.2 , respectively.

There was a significant correlation between Ki-67 and p53 ($R = 0.82$, $p < 0.001$) and an inverse relationship between survival and both Ki-67 ($R = -0.67$, $p < 0.001$) and p53 ($R = -0.64$, $p < 0.001$) rates (Figure 1). No significant correlation was found between survival and age ($R = 0.22$, $p = 0.22$) or CEA ($R = 0.08$, $p = 0.67$). Similarly, there was no relationship between CEA and age ($R = 0.34$, $p = 0.06$), Ki-67 ($R = -0.02$, $p = 0.90$) or p53 ($R = 0.03$, $p = 0.87$) rates.

Discussion

Ki-67 is a nonhistone nuclear protein closely associated with proliferating cells (12). The adverse prognostic value of Ki-67 overexpression has been studied in different malignancies, especially breast cancer and lung cancer (13, 14). In addition, in patients with breast cancer, a positive relationship between Ki-67 and tumor response to neoadjuvant chemotherapy has been observed (4, 15).

In patients with CRC, high Ki-67 expression is usually associated with a higher histological grade of the tumor, lymph node involvement and shorter disease-free interval (5, 12). Several studies suggest that the expression of Ki-67 antigen is a useful indicator of survival of patients with CRC, and an adverse prognostic value of high Ki-67 expression after curative resection for CRC has been shown (16, 17).

We found an inverse correlation ($R = -0.67$, $p < 0.001$) between overall survival and the percentage of Ki-67-positive tumor cells and our results were consistent with these studies. However, an improved outcome of patients with stage II and III CRC and Ki-67 overexpression has also been reported, independent of adjuvant chemotherapy, suggesting

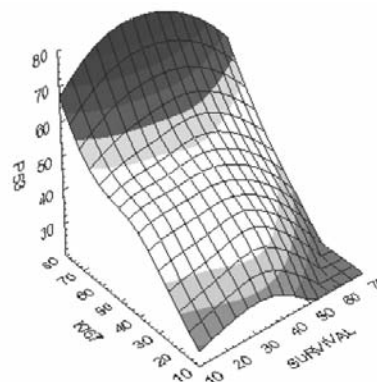


Figure 1. Relationship between Ki-67 (%), p53 (%) and survival (months).

that proliferating malignant cells are more vulnerable to chemotherapy-induced tumor cell death (7, 18).

The protein p53 is a nuclear phosphoprotein encoded by the *TP53* gene, a tumor-suppressor gene that is implicated in controlling DNA repair and cell-cycle regulation (5, 19). p53 controls the induction of growth arrest at cell cycle checkpoints and apoptosis and it has been hypothesized that the basis of its suppressor function is to eliminate damaged cells (20, 21). The expression of desmocollin 3 (DSC3), a member of the cadherin superfamily involved in carcinogenesis, is regulated by p53 and the methylation of DSC3 DNA represents a prognostic marker in CRC (22). Moreover, in normal cells, p53 binds to the murine double minute-2 (*MDM2*) oncogene, which prevents its action and promotes its degradation by acting as a ubiquitin ligase (5, 19). Although in some studies, p53 overexpression did not have significant prognostic value in the adjuvant setting of CRC (23), in most studies, the accumulation of p53 protein identified patients at higher risk of tumor progression (6, 24, 25).

Our results confirm the inverse relationship between p53 overexpression and survival. Serum levels of the autoantibody to p53 do not have prognostic significance in long-term follow-up of patients with CRC (26).

Conclusion

Our preliminary results showed that both Ki-67 and p53 overexpression in CRC is associated with a worse outcome. However, in this selected group of patients, these prognostic markers were independent of age, and the preoperative CEA serum levels did not have any relationship with survival.

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