Abstract  Tumour growth is regulated by a balance between proliferation, growth arrest and programmed cell death (apoptosis). Until recently, the majority of the studies dealing with oncogenesis has been focused on the regulation of cell proliferation. There is now growing understanding that control of growth arrest and apoptosis play key roles in the development of human cancer and in cancer treatment. Some of the more heavily studied proteins of importance for the control of growth arrest and apoptosis are p53, p21, bcl-2 and bax. Alterations in the p53 protein may lead to malignant transformation and defect therapy response, most likely as a result of defective p53-dependent apoptosis. In addition, p21 (WAF1/CIP1) is involved in cell-cycle arrest and probably in induction of p53-dependent apoptosis. Proteins belonging to the bcl-2 family are also important for normal apoptosis. Overexpression of bcl-2 protein is thought to reduce the apoptotic capacity, while bax protein seems to be necessary for induction of apoptosis. In this study, we have immunostained tissues from 93 primary colon carcinomas and have examined the expression of p53, p21 (WAF1/CIP1), bcl-2, bax, pRb and cyclin D1 for evaluation of their roles in colon-cancer progression. A highly significant association between p53 accumulation and downregulation of p21 (WAF1/CIP1) was seen. We also found a strong association between reduced/absent p21 and the development of metastases and death due to cancer disease. Cyclin D1, bcl-2 and bax protein failed to have independent prognostic impacts. Bcl-2 and bax protein levels showed an inverse relationship. The results of the present study indicate that reduced p21 protein levels play an important role in progression of colon cancer. We concluded that evaluation of p21 expression in primary colon carcinomas at the time of surgery might be a valuable tool in defining patients with a high risk of developing metastases.

Key words  Apoptosis · bax · bcl-2 · Colon carcinoma · CyclinD1 · Immunohistochemistry · p21 · p53 · pRb

Introduction  Apoptosis of tumour cells is necessary and desirable when chemotherapy treatment is given, and resistance to apoptosis plays an important role in tumours that are refractory to chemotherapy and ionising radiation. Factors affecting the apoptotic function by any mechanism may also interfere with the prognosis of cancer patients. Many of the genes involved in the induction and inhibition of apoptosis have been mapped [16]. Both p53-dependent and p53-independent pathways seem to be of importance [4]. One of the main functions of p53 after DNA damage is the induction of transcription of effector genes, such as p21 (WAF1/CIP1) [20]. p21 binds cyclin-dependent kinases in the G1 phase of cell cycle and inhibits their ability to phosphorylate other proteins like pRb (which is necessary for cell-cycle progression) [10]. The temporary growth arrest gives sufficient time for DNA repair prior to DNA synthesis. p53-Protein abnormalities (alone or in combination with defects in critical downstream effector proteins) can lead to altered cell-cycle growth arrest, deficient DNA repair and apoptosis. Two other proteins that seem to play a significant role in apoptosis are bcl-2 and bax. Bcl-2 has been shown to block both p53-dependent and -independent cell-death pathways [6, 18], while bax increases the apoptotic capacity of the tumour cells [17]. Another family of proteins that interfere with cell survival and cell division are D-type cyclins. The D-type cyclins (D1, D2 and D3) are important in regulating the G1 checkpoint [11]. The human cyclin-D1 gene (CCND1) is located on chromosome band 11q13; the cyclin-D2 gene (CCND2) is on 12p13, and the cyclin-D3 gene (CCND3) is on 6p21 [12]. Cyclins D1, D2 and D3 promote progression
through the G1 phase of the cell cycle by regulating the activity of the cyclin-dependent protein kinases (CDKs) Cdk4 and Cdk6. In their activated forms, these kinases are capable of phosphorylating the retinoblastoma protein pRb, a critical target of G1 CDKs that is also thought to be of importance for the stability of D-cyclin kinases [23].

The role of these proteins in the evaluation of patient prognosis has been elucidated in some studies. Caffo et al. [3] demonstrated that the presence of p21 protein in tumour cells is a marker for chemotherapy response in a study of breast-cancer patients. In a previous study of breast-cancer patients, we demonstrated that overexpression of bcl-2 is associated with downregulation of p21 in tumours with normal p53 protein [1]. Recently, Zhan et al. [24] also showed that increased expression of bcl-2 in human Burkitts-lymphoma WMN cell line is capable of p21 suppression. Abnormal expression of the retinoblastoma protein (pRb) and cyclin D1 have been reported in a variety of malignancies, but their frequency and prognostic impact in colorectal cancer have been evaluated in only a few studies [15, 22].

In this study, we analysed the protein expression of p53, p21, cyclin D1 pRb, bcl-2 and bax in human colon carcinomas. Thus, the aim of the study was to explore apoptotic (p53, p21, bax) and anti-apoptotic (bcl-2, cyclin D1) potentials in colon-carcinoma patients.

Materials and methods

Material for this study was obtained from 93 patients with primary colon carcinoma admitted to Central Hospital of Akershus between 1988 and 1990. The mean age at diagnosis was 76.7 years (range: 45–89 years). Forty-three patients were classified as adenocarcinomas, 30 as Dukes B, 30 as Dukes C and 20 as Dukes D. The immunostaining results are presented in Table 2.

Table 1. The amounts of immunopositive cells were estimated and the sources and dilutions of the antibodies are shown in Table 1. The amounts of immunopositive cells were estimated semiquantitatively: grade “+” corresponds to 5–10%, grade “++” to 10–50%, and grade “+++” to more than 50% positive cells. All series included positive and negative controls. The results of control staining were satisfactory.

Statistical methods

Statistical analysis was performed using the χ² test. The level of statistical significance was defined as P<0.05.

Results

The immunostaining results are presented in Table 2. P53-Protein expression was detected in 72 tumours (77.4%; Fig. 1A). Sixteen tumours showed protein expression in 5–10% of the cells (+), three tumours showed protein expression in 10–50% of the cells (++) and 53 tumours showed protein expression in more than 50% of the cells (+++).

p21 (WAF1/CIP1) immunoreactivity was detected in 15 (16.1%) of the tumours (Fig. 1B). Only nine of these showed strong immunoreactivity (++/+++), and six were immunoreactive in 5–10% of the tumour cells (+).

When bcl-2 protein immunoreactivity was evaluated, we detected protein expression in 23 (24.7%) of the tumours (Fig. 1E). Ten of these 23 cases showed immunoreactivity in 5–10% of the cells, 11 in 10–50% of the cells and two in more than 50% of the cells.

Immunoreactivity for bax protein was detected in 87 (93.5%) of the tumour samples (Fig. 1F). The immunostaining was abundant in 80 of these (+++/+++), while, in seven of the samples, bax-protein immunoreactivity was detected in 5–10% of the tumour cells (+). In some samples immunostaining was also seen in tumour cell nuclei.

Only eight samples (8.6%) showed immunoreactivity for cyclin D1 (Fig. 1C). Four of these were strongly immunoreactive (++/+++), while the other four showed immunoreactivity in only few of the tumour cells (+). Strong pRb-protein reactivity was detected in all samples (Fig. 1D).