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THE SIGNIFICANCE OF KEY REGULATORS OF APOPTOSIS IN THE DEVELOPMENT AND PROGNOSIS OF PROSTATE CARCINOMA. I. PROTEINS OF THE BCL-2 FAMILY AND PROTEIN P53

Jana Knillová*, Zdeněk Kolář

Laboratory of Molecular Pathology and Institute of Pathology, Faculty of Medicine, Palacký University, 775 15 Olomouc, Czech Republic, e-mail: madarova@tunw.upol.cz

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The molecular basis for the transition of carcinoma of the prostate from androgen-dependent to androgen--independent growth is largely unknown. Currently for example, it is not clear whether the androgen-independent phenotype is a result of selection of a subgroup of genetically distinct prostate tumour cells which are already hormone-resistant or a genetic adaptation of prostate tumour cells to the hormone therapy itself. It has also been established that prostate tumour transformation is a result of homeostatic control defects, a line of thinking directed toward elucidating the apoptotic profile of prostate tumour cells that may be important in determining prognosis, response to therapy and illness progression. Main consideration in this part of rewiev is given to the role of Bcl-2 and members of the Bcl-2 family, and tumour suppressor gene p53.

INTRODUCTION

The incidence and prevalence of cancer of the prostate is steadily rising and on the basis of the number of new cases, prostate neoplasms are the fourth most serious tumour illness in men[1]. The highest incidence is described for the USA while in the Czech Republic it occupies third place after lung and colorectal cancer in men[2]. Treatment of prostate carcinoma also presents a serious medical problem: surgical therapy is accessible only for localised tumours. Relapsing, locally spreading and metastatic forms are inoperable and patients are then usually subjected to androgendeprivation therapy. In the absence of androgen, tumour prostate cells in androgen-dependent tumours undergo apoptosis in the same way as normal prostate cells[3, 4]. For this reason apoptosis induction has therapeutic significance in the treatment of prostate cancer. Antihormone therapy lowers the level of circulating androgens or blocks the effect of male sex hormones at the level of androgen receptors (AR). Tumour growth following this therapy is unfortunately only temporarily slowed down and androgen-dependent tumours shift to androgen-independence. In the latter, chemotherapy or ionising radiation can also achieve apoptotic pathway destruction of cancer cells, but the majority of these tumours are resistant to conventional chemo and radiotherapy[5]. It is therefore of paramount importance to understand the transition from androgen-dependence to androgen-independence.

The molecular basis of this transition however is largely unknown. Currently for example, it is not clear whether the androgen-independent phenotype is the result of selection of a subgroup of genetically distinct prostatic tumour cells which are already hormone resistant or a genetic adaptation of prostatic tumour cells to hormone therapy[6–8]. A model of prostate cancer development is shown in Fig. 1. Molecular mechanisms that enable tumour cells to survive in a low androgen environment probably include multiple gene alterations, among which are alterations of AR (amplification, mutation and ligand-independent activation), activation of oncogenes, changes in the genes regulating the cell cycle and last but not least, alterations in the genes participating in the regulation of apoptosis[10–15]. Fig. 2 shows a proposed scheme of genetic events in prostatic cancerogenesis. The finding that tumour growth in the prostate gland is dependent on exit from normal mechanisms of the cell cycle control is directing research into elucidating the mechanisms of prostate tumour cell apoptosis and/or proliferation which may be important determiners of prognosis, treatment response and illness progression. This theme forms the substance of the present review. Main consideration is given here to the role of Bcl-2 and members of the Bcl-2 protein family, the products of tumour suppressor genes p53, pRb and PTEN and also the natural inhibitors of cyclin dependent kinases – proteins $p21^{Waf1/Cip1}$ (p21) and $p27^{Kip1}$ (p27). Attention is also given to the FAS-mediated pathway.

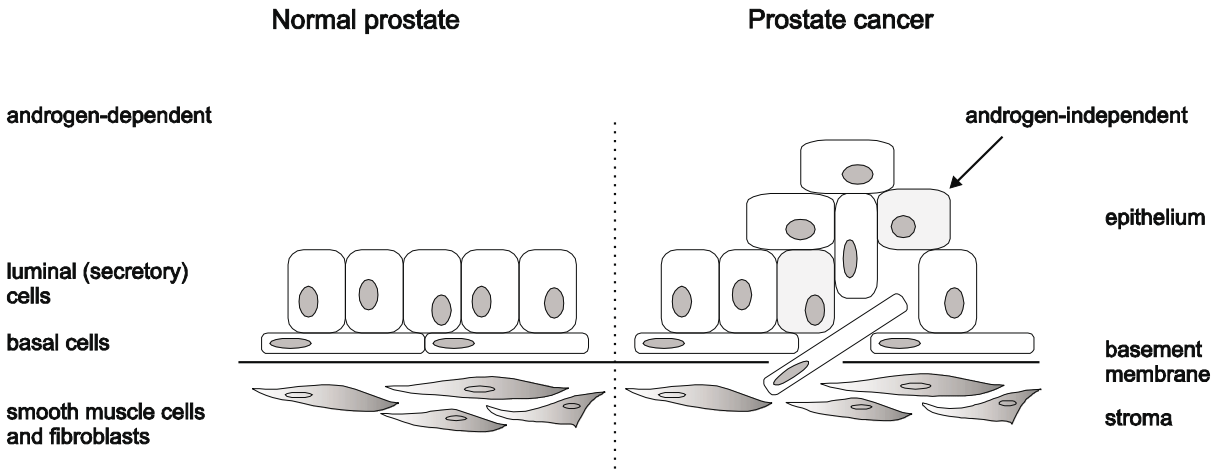

Fig. 1. Model of prostate cancer development (adapted from Craft and Sawyers[8]). The histologic features of the normal prostate gland include stroma cells, basement membrane, basal epithelial cells and secretory cells. The secretory epithelial cells express PSA and are dependent on androgen for survival. The basal epithelial cells do not express PSA, do not require androgen for survival, but respond to androgen post-castration. Prostate cancer cells express secretory differentiation markers such as PSA and are dependent on androgen for growth but not survival. Under the selective pressure of androgen ablation therapy, a subclone of cancer cells emerge which is no longer dependent on androgen for growth or survival. This subclone is responsible for hormone-refractory disease.

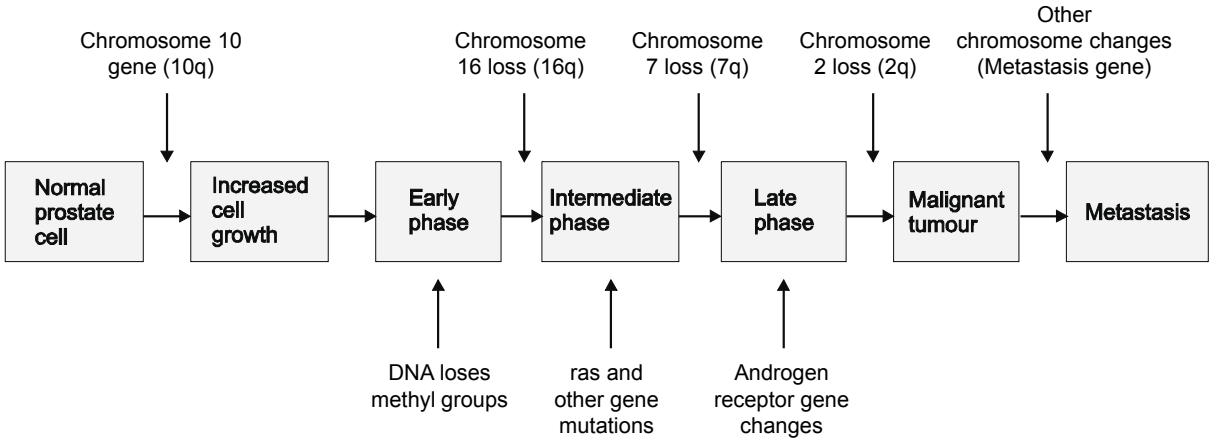

Fig. 2. Proposed scheme of genetic events in prostatic carcinogenesis (adapted from Coptcoat[11]).

Bcl-2 FAMILY

Members of the Bcl-2 protein family are key apoptosis regulators[16]. Protein Bcl-2 was discovered during studies of the t(14;18) chromosome translocations often found in non Hodgkin's lymphoma and follicular lymphoma which are characterised by upregulation of Bcl-2 gene expression and resistance to apoptosis induction[17]. The Bcl-2 family of proteins consists of both inhibitors and promoters of programmed cell death. In mammals not less than 15 members of the Bcl-2 family have been identified. Others have been found in viruses. Among antiapoptotic proteins are Bcl-2, Bcl-X_L, Mcl-1, Al and Bcl-w. Proapoptotic proteins include Bax, Bak, Bcl-X_S, Bad, Bik and Bid. Best described are Bcl-2 and Bax[16, 18, 19]. Each family member has at least one out of four evolutionarily conserved motifs, known as Bcl-2 homology domains (BH1-BH4)[19-21]. Proapoptotic and antiapoptotic family members can form heterodimers and homodimers and their relative concentration determines the apoptotic potential of cells[16, 22]. For example Bcl-2 can form homodimers with different molecules of Bcl-2 and/or heterodimers with Bax molecules or other family members. The prevalence of Bax determines apoptosis, while the excess of Bcl-2 blocks it. Overall these findings, the mechanisms regulating apoptosis in the case of proteins Bcl-2 and Bax remain unclear. While dimerisation seems to be important, the significance of heterodimerisation

is still controversial and recently, studies have shown that both Bcl-2 and Bax can regulate the ability to commence apoptosis independently[23, 24].

Expression of Bcl-2 has been described in many epithelial malignancies including that of the prostate gland[18, 25–28]. It has been confirmed that elevated levels of Bcl-2 protein can contribute to the progression of prostate cancer, to the development of metastasis, and to the hormone-insensitive stage[25, 29]. The participation of Bcl-2 in multistep carcinogenesis of the prostate has been shown by in vivo experiments in transgenic mice[30]. The role of Bcl-2 in tumourigenesis may be in one of two ways: it can participate in apoptosis suppression and or in stimulating tumour angiogenesis[31]. In normal prostate tissue Bcl-2 expression is limited to basal cells of the glandular epithelium. These are resistant to the effects of androgen deprivation. In contrast, secretory epithelial cells, which have no detectable Bcl-2 expression, undergo apoptosis following androgen deprivation[5, 8, 32, 33]. A strong association between Bcl-2 expression and the development of androgen-independent prostate cancer was described by McDonnell et al. in 1992[32]. Since then several other studies have confirmed that androgen-independent prostatic tumours are typically immunoreactive to Bcl-2 protein[12, 26, 33].

A negative correlation between Bcl-2 expression and clinical prognosis of non-metastasising prostate tumours has also been shown in patients undergoing radical prostatectomy[12, 34, 35]. Szostak et al. published the first study showing a negative correlation of apoptosis induction and prognosis with Bcl-2 overexpression in patients with cancer of the prostate treated using brachytherapy. They described a significantly raised Bcl-2 expression in patients in which therapy failed, in comparison with those who responded[36]. The increase of Bcl-2 expression has been described in patients with locally spread or metastatic forms of prostate cancer treated using hormone ablation therapy[28]. For this reason Bcl-2 expression is a negative prognostic indicator in these patients[5]. It seems that Bcl-2 enables prostate tumour cells to survive after castration and that hormone deprivation therapy may select Bcl-2 positive cells which do not react to lowered levels of hormone by undergoing cell death[12].

Hering et al. compared the frequency of positive Bcl-2 expression in adenocarcinomas of the prostate with low and high Gleason scores. They found that higher expression of Bcl-2 in tumour samples was observed in association with more advanced Gleason scores and proposed that an increase in the ratio of this antiapoptotic protein often occurs during the progression to prostate cancer[29].

In vitro studies using prostate cancer cell lines confirm the role of Bcl-2 in inhibition of apoptosis and in the progression to androgen-independence. Studies have shown that prostate cancer cells that overexpress Bcl-2 are more resistant to apoptosis induction after hormone ablation in vivo and in vitro[37, 38]. In addition, Bcl-2 expression in prostate tumour lines is associated with lowered apoptotic response after treatment with different chemotherapeutics, ionising radiation and resistance to apoptosis caused by nutritional deprivation. These data confirm that the antiapoptotic function of Bcl-2 is oncogenic and contributes to the resistance to antiandrogen treatment[18, 39, 40]. Baltaci et al. determined the occurrence of Bcl-2 protein expression in low and in high grade prostatic intraepithelial neoplasia (PIN) lesions and investigated thus the role of Bcl-2 in tumourigenesis of the prostate. They reached the conclusion that Bcl-2 protein expression is associated with early tumourigenesis of the prostate [41]. On the other hand, according to Diaz at al., overexpression of Bcl-2 seems to be a late and unrelated molecular event to tumourigenisis[42].

Krajewska et al. described Bcl-2, Bax, Bcl-X_L and Mcl-1 expression in primary and metastatic prostate carcinomas. High grade carcinomas and metastasis expressed antiapoptotic proteins more frequently and with higher intensity than lower grade tumours. In contrast, proapoptotic protein Bax was expressed in each tested sample regardless of tumour grade[27]. It has also been shown in cell lines that lowered sensitivity to the effects of cytotoxic chemotherapy is influenced by overexpression of the antiapoptotic member of family Bcl-X_L protein[43]. Resistance to apoptosis in the absence of proapoptotic Bax has also been described[44]. It has been demonstrated that the absence of Bax may be caused by higher degradation of the protein by the ubiquitin/proteasome pathway. Higher levels of Bax degradation correlate well with lower levels of Bax protein and higher Gleason score[45].

Studies using antisense oligonucleotides directed against bcl-2 mRNA in different chemoresistant cells showed a restoration of chemosensitivity to chemical compounds[18, 46]. A similar approach using ribozymes (enzymes connected with antisense nucleotides) against blc-2 and bcl-X_L also promoted apoptosis[47, 48]. These observations show that ectopic Bcl-2/Bcl-X_L expression leads to a death resistant phenotype. This finding also provides new impetus to the development of potential treatment. Antisense strategies aside, therapies influencing Bcl-2/Bcl-X_L protein configuration can be used and in this way cause inhibition of homo- and heterodimerisation for example by phosphorylation of Bcl-2. In cell lines derived from prostatic carcinoma, Bcl-2 is phosphorylated by antitumour substances causing a break in production and in the stability of microtubules (taxol, vinkristin, kolchicin and others). Cell lines with no Bcl-2 expression are resistant to taxol and similar substances. Bcl-2 phosphorylation is achieved in the G2/M phase of the cell cycle, where a blockade occurs in polymerisation and depolymerisation of cell microtubules[49–51]. The above mentioned studies have also shown that post-translational phosphorylation of protein Bcl-2 by the substances mentioned is induced in the serine position. A further possibility for influencing protein configuration is for example the use of the BH3 peptides and upregulation of the expression of death promoters Bax, Bak and others[18, 52].

From the above cited in vitro and in vivo studies, it follows, that Bcl-2 expression operates against apoptosis induced by androgen deprivation and cytotoxic chemotherapy. It is evident that inhibition of the death antagonists from the Bcl-2 family could significantly promote apoptosis. Intensive study of these groups of proteins is leading to attempts to modulate their expression and function and thus provide possible treatment goals for this cancer. Some current approaches have been mentioned and theoretically it is possible to propose an approach using combined therapies which would effectively upregulate death promoters through gene therapy and inhibit the death suppressors as for example in the case of chemotherapeutically induced phosphorylation of Bcl-2[18].

p53

Tumour suppressor gene p53 codes the transcription factor which is activated in response to a wide spectrum of stress signals. Protein p53 monitors genomic integrity and in cases of DNA damage it has the ability according to the severity of the injury to induce arrest in the G1 phase of the cell cycle, when the cell acquires time for DNA repair, or in the case that the gene damage is irreparable, to induce apoptosis. Protein p53 is also involved in control of the G2/M checkpoint. Activated p53 stimulates transcription of downstream genes whose products facilitate the adaptative and protective activity of the cell. Following DNA damage the stability of p53 protein also increases, probably as a result of phosphorylation[53–55]. Identifying the p53 responsive genes regulating these processes is still a question of wide interest. The specific sequences of DNA able to bind p53 are created by two repetitions at 10 bp. In tumours, mutated forms of p53 are usually found. These lack the ability to bind to DNA or activate transcription. It has been shown that for antigrowth and antitumour activity unimpaired p53 transcription activity is necessary[54, 56]. One of the most important events for G1 suppression of the cell cycle in response to genotoxic damage is p53 dependent transcription regulation of $p21^{Waf1/Cip1}$. In this view $p21^{Waf1/Cip1}$ serves as an effector of the cell cycle arrest in response to activation of the p53 checkpoint pathway[57-59]. Further critical target genes are gadd45 and 13-3-3σ. These control transition through the G2/M phase. None of these genes however has any connection to p53 dependent apoptosis[60–62]. The path which assists p53 influenced apoptosis represents both the transcription regulation of target genes and also a transcription independent function of p53. This may reflect different mechanisms by which p53 operates in different cell types. Apoptosis dependent on p53 is induced via the pathway Apaf-1/caspase 9. Caspase 9 is concerned with the regulation of mitochondrial cytochrome c release. A range of genes regulated by p53 has been identified. These contain a p53 responsive element and it has been found that some of them mediate p53 dependent apoptosis. These genes include Bax, CD95 (Fas/Apo-1), killer/DR5, Ei24/Pig8, Noxa, PERP, p53 AIP and PUMA. Some studies suggest also a potential connection between p53 and Rb in the regulation of the cell cycle, apoptosis and tumour progression[56, 62–64].

p53 abnormalities are among the most frequent molecular abnormalities in human tumours. The gene for p53 is localised on chromosome 17. In tumours of the prostate loss of heterozygosity (LOH) on chromosome 17 appears in close to 20 % tumours[65]. Around 50 % of tumours carry mutation in p53 and this inhibits its tumour suppressor function. Most frequent are, as in the case of other tumours, mutations in the central DNA binding domain. Mutations in other domains have also been identified. Mutations of p53 gene typically lead to an extended half life of the p53 protein. This renders its detection possible using immunohistochemical methods, while wild type under these conditions is detected with difficulty. These mutations usually lead to loss of p53 function, subsequent to defects in the cell cycle control and changes in genome integrity[12, 66, 67]. However, the described incidence of p53 mutations in localised tumours of the prostate varies. Frequencies of between 47–80 % or 1–42 % have been reported[5, 68]. In developed hormonally unresponsive tumours the frequency is raised up to 94 %[69]. Differences in the data are likely for a variety of factors. Apart from the influence of technical aspects, fluctuations in the described incidence of p53 alterations also follow from heterogeneity in topographical distribution of the mutations[68, 70, 71]. Mirchandani et al. describe multifocal tumours within the prostate that appear to differ in their propensity to harbour the mutant gene and that there is also intratumour heterogeneity[70]. Most studies show a low percentage of p53 abnormalities in early stages of carcinoma and their increase in frequency during the progression of the illness, the highest being in patients with hormone-nonresponsive tumours[69]. Mutations in the p53 tumour suppressor gene are generally seen as a late event in progression of prostate cancer and are associated with androgen-independence, metastasis and poor prognosis. Leite et al. concluded that, although p53 mutation is a rare event in prostate carcinogenesis, the detection of p53 protein by immunohistochemistry is common[72]. In this case it is associated with decreased apoptosis, increased histological grade and tumour stage. The last named authors have also shown that overexpression of MDM2 has a role in prostate carcinogenesis and is frequently detected in these tumours. It is also associated with higher cell proliferation and greater prostate tumour volume. Indeed, it has been suggested that MDM2+/p53+ phenotype identifies a prostate tumour with aggressive behaviour. On the other hand, relatively high levels of p53 mutations are also found in normal prostate tissue in patients with cancer of the prostate, PIN and benign prostatic hyperplasia (BPH)[71]. Owing to this variability the significance of p53 mutations in prostate cancer remains controversial.

Most studies have shown that p53 protein expression in cancer of the prostate serves as an unfavourable indicator[12, 73]. However, other authors argue that p53 may not be useful as a prognostic marker in most patients with prostate cancer[68, 74]. The predictive value of p53 emerges from the correlation of p53 protein expression with higher Gleason score, pathological stage and proliferation in localised primary tumours of the prostate. The number of p53 mutations is increased in progressed tumours, with a higher incidence in tumours of the androgen-independent type as well[5, 75]. Yang et al. have shown that p53 immunoreactivity in localised tumours of the prostate correlates with higher incidence of relapse compared with comparable lesions which lack p53 mutation[76]. Quinn et al. report similar results[77]. In cases of locally advanced non-metastasising stages of cancer, p53 immunoreactivity after radical prostatectomy improves the predicted outcome. The addition of this variable to those routinely determined may identify a subset of patients who may benefit from more intensive postoperative surveillance and adjuvant therapy[78]. Also according to Byrne et al. p53 mutations contribute to progression of prostate cancer and the time period of tumour progression in patients with positive p53 is significantly shorter than those with p53 negative[79]. Proliferative activity determined using PCNA expression in prostate carcinomas is usually low but this correlates with positive p53 immunostaining[72]. A positive correlation between Ki 67 and p53 protein expression has also been found[80] but other findings contradict this. A number of researchers have found no significant correlation between p53 overexpression, p53 mutations, clinicopathological parameters and survivability[81, 82]. Brewster et al. on the basis of biopsy samples discovered that aberrant p53 is associated with significantly poorer prognosis after radical prostatectomy than normal p53[35]. In contrast Stackhouse et al. contest the usefulness of p53 analysis in biopsy samples[83].

No relation between p53 and Gleason score, for example has been found in further examination of biopsy material[84]. A series of studies has shown heterogeneity of p53 expression in primary tumours. There is evidence that p53 mutations at the primary site of these tumours may be predictive of metastases. There are also data supporting the hypothesis that p53 dysfunction within prostate cancer may exist in foci of tumour cells that are clonally expanded in metastases[77, 85, 86]. Cheng et al. showed that assessment of biologic changes including p53 alterations in regional lymph node metastases could be of value in assessing the biological aggressiveness of prostate carcinoma, whereas p53 expression in the primary tumour does not appear to influence patient outcome[81].

Alterations in the p53 gene may play a role in the development of radiation resistance. Despite the conflict in the data, genetic alteration in p53 can be used as a pretreatment marker in predicting local treatment failure with ionising radiation therapy[68, 87–89]. Induction of p53 protein after radiation shows specific dependence on cell type of prostate tissue. Epithelial cells derived from normal prostate, BPH and adenocarcinoma show no evidence for p53 accumulation 1–3 hours following radiation but in stromal cells in the same patients a higher level of p53 was noted. In addition, higher $p21^{Waf1/Cip1}$ expression was observed only in stromal cells. To date, the significance of these findings in relation to radiation is not clear[90]. Apart from resistance to radiation, mutations in the p53 gene are also associated to resistance to certain chemotherapies[91].

In patients with p53 mutation, apoptosis induction following androgen ablation may also be blocked[68]. Higher expression of mutated p53 was noted in patients in whom antiandrogen therapy was ineffective[92–94]. Studies show that neo-adjuvant hormonal therapy may cause selection of minor p53 mutated clones, rather than the induction of wild-type p53 and that positive p53 immunostaining correlates with AR gene amplification[94, 95]. These results suggest that inactivation of the p53 gene may lead to genetic instability in a subset of prostate carcinomas enabling them to achieve properties, such as AR gene amplification, that allow them to grow in low levels of androgen and therefore cause tumour progression[95]. Apakama et al. have shown that combined detection of p53 accumulation and Bcl-2 overexpression may be useful in predicting hormone resistance. Alteration in these genes causing deregulation of programmed cell death may prevent response in patients on androgen ablation or cause escape from hormone control[26].

The levels of p53 mRNA and p53 protein were shown to increase in the regressing rat ventral prostate following castration. This enhanced p53 expression appears to be associated with return of prostatic epithelial cells to a defective cell cycle and may be important for subsequent induction of apoptosis[12, 96]. Further studies using knockout mice show that p53 is not essential for apoptosis in the prostate after castration. Thus the p53 gene may play a key role in apoptotic response depending on cell type, and both normal and malignant prostate cells may undergo apoptosis by p53 – independent pathways[5, 97, 98]. The contribution of p53 inactivation to resistance of prostatic epithelial cells to the effects of apoptosis induced by androgen deprivation remains highly controversial.

The assumption that parallel assessment of p53 and Bcl-2 expression in biopsy before treatment can be useful for predicting response to radiotherapy has not clearly been confirmed[99]. Biomarkers p53 and Bcl-2 appear to be important in predicting the recurrence of prostate cancer when prostatectomy specimens were analysed but this usefulness was not apparent with immunohistochemical staining of prostate biopsies[83]. Biopsies also failed to show any relation between p53 and Bcl-2 expression and Gleason score[84]. Cesinaro et al. analysed p53 and Bcl-2 expression in diagnostic biopsies and in prostatectomies carried out after neoadjuvant hormone therapy, in order to determine their role in hormone resistance. After hormone therapy there were no changes in Bcl-2 expression and no correlation was found be-

tween Bcl-2 and clinicopathological variability either in the biopsies or in the prostatectomies[94]. These findings contradict those of Tsuji et al. however[28].

The potential contribution of other members of the p53 gene family in regulating cell death in the prostate is not well known[12, 100]. Mutations of genes p51 and p73 have not been found in prostate tumours. Protein p73 is expressed in both normal and tumour tissues. In contrast to normal and preneoplastic prostatic tissue, the vast majority of prostate adenocarcinomas do not express p63. This shows that p63 may protect epithelial cells against neoplastic transformation and that p63 immunohistochemistry represents a potential novel adjuvant method for facilitating diagnosis of prostate cancer using prostate needle biopsies. Both of these genes may have roles in addition to tumour suppression[101–103].

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