

Volume 12, issue 4, December 2001 | Articles originaux

Immunoexpressions of p21, Rb, mcl-1 and bad gene products in normal, hyperplastic and carcinomatous human prostates

Abstract

A comparative study of the expression of p21, Rb, mcl-1, and bad gene products, which are involved in the control of the cell cycle, was performed in normal, hyperplastic, and carcinomatous human prostates by means of a semiquantitative immunochemical study. This included Western blot, ELISA, and immunohistochemistry procedures. In normal prostates, immunoexpression of

the four gene products was scanty or absent. In men with benign prostatic hyperplasia, immunoreactions to the four proteins studied were found in many epithelial cells and some stromal cells. In prostatic carcinoma, the immunostaining pattern was as in hyperplastic prostates but the numbers of both epithelial and stromal cells were higher. Present results indicate that immunoexpression of p21, Rb (both the phosphorylated and dephosphorylated forms), mcl-1, and bad gene products are markedly increased in prostates with proliferative alterations but that these proteins do not discriminate between benignant (hyperplasia) and malignant (adenocarcinoma) prostatic tumours, although immunoexpression is higher in prostatic carcinoma.

Full text

INTRODUCTION

The processes of both cell survival and cell death involve highly regulated signalling pathways that are currently the subject of intense investigation. The rates of epithelial cell growth and death in the adult normal prostate gland are in equilibrium [1]. At present, the signalling pathways that lead to apoptosis or cell growth are beginning to be defined, and a number of proteins have been identified.

The p21 waf1/cip1 a tumour suppressor gene has been identified and cloned [2], and is transcriptionally activated by p53 in response to DNA damage [3]. The 21 kDa product of this gene (p21) is a cyclin-dependent kinase inhibitor [4], responsible for inactivating the retinoblastoma protein (Rb) [5], and able to arrest the cell cycle at the G1 phase by inhibiting DNA replication through interaction with proliferating cell nuclear antigen PCNA [6]. Immunohistochemical location of p21 in normal prostate and benign prostatic hyperplasia (BPH) is controversial. While Aaltomaa *et al.* [7] found no reaction, Shiraushi *et al.* [8] and Baretton *et al.* [9] described occasional positive p21 immunoreactivity in non-neoplastic prostates. Different immunohistochemical studies have reported the presence of this protein in the nuclei of the malignant prostate tissue [7, 8].

Retinoblastoma protein (Rb) is a 110 kDa phosphoprotein that plays a central role in human cancer because regulates the transition between G1 and S phases in the cell cycle [5]. When Rb is phosphorylated by the cyclin-dependent kinases

cdk-2 and cdk-4, it induces the entry of cells into the cycle [10]. But dephosphorylation of Rb during G1 progression results in arrest of the cell cycle [11]. This dephosphorylation is caused by overexpression of p21 that inhibits the cyclin-dependent? [12]. Rb has been detected in BPH [13] and prostate cancer [13, 14].

The Bcl-2 family is a growing gene family that play a major role in regulation of apoptosis, some genes acting as inhibitors (bcl-2, mcl-1, bcl-X1) or promoters (bax, bad, bak and bcl-Xs) of apoptosis [15]. Membership of the family was first defined by a homology in two conserved regions: BH1 (Bcl-2 Homology) and BH2 [16]. More recently, two additional domains, BH3 and BH4 have also been recognised [17]. Bad (the Bcl-2 associated death promoter) is a BH3 member of this family, and its function is regulated by phosphorylation in response to survival factors such as NGF, IGF-1 and IL-3 [18]. Bad forms heterodimers with anti-apoptotic bcl-2 homologues such as bcl-2 and bcl-X_L, neutralising their protective effects and promoting cell death [19]. In normal prostate, negative results were reported by Kitada *et al.* [20], who used immunohistochemistry analysis. To our knowledge there are no reports on bad immunoexpression in prostatic disorders.

Mcl-1 (myeloid cell leukaemia-1) is an antiapoptotic, bcl-2 family protein with Mr 37 kDa, described as an early induction gene during myeloblastic leukaemia cell differentiation [21]. This antiapoptotic protein is up-regulated by cell survival cytokines such as macrophage colony-stimulating factor, interleukin-1beta and interleukin-3 [22]. Krajewski *et al.* [23] described moderate mcl-1 immunostaining in non-pathological epithelial prostate cells. In prostate cancer, mcl-1 is expressed at higher levels in the epithelial cells [24]. No previous references to mcl-1 have found in BPH.

The present report concerns a comparative immunohistochemical, ELISA and Western blot study of p21, Rb, bcl-2 and mcl-1 in normal prostates, BPH and prostatic carcinoma in order to investigate the relationship between these proteins and their relationships with the apoptosis-proliferation equilibrium.

MATERIALS AND METHODS

The materials used included: (a) histologically normal prostates obtained at autopsy (8-10 hours after death) from 15 men (aged from 20 to 38 years) without

histories of reproductive, endocrine or related diseases; (b) prostatic biopsies from 25 men (aged from 53 to 88 years) diagnosed clinically and histopathologically with benign prostatic hyperplasia (BPH); and © prostatic biopsies from 30 men (aged from 54 to 69 years) diagnosed with prostatic cancer (PC, dominant Gleason grade 3, Gleason score 5-7). The patients received neither hormonal therapy before prostatectomy nor were diagnosed for metastatic cancer. Each sample was divided into three samples: one that was immediately processed for immunohistochemistry, and the other two samples were frozen in liquid nitrogen and maintained at - 80° C for ELISA and Western blot analysis. Removal of tissues and study of autopsy samples were approved by the Ethics Committee of the Hospital, and performed with the consent of the patients' relatives.

For Western blot analysis, tissues were homogenised in the extraction buffer (0.05 M Tris-HCl, pH 8) with the addition of a cocktail of protease inhibitors (10 mM iodoacetamide, 100 mM phenylmethyl sulphonic fluoride, 0.01 mg/ml of soybean trypsin inhibitor, 1 mM benzamidine and 1 mul/ml of leupeptin) and phosphatase inhibitors (10 mM sodium fluoride and 1 mM sodium orthovanadate) in the presence of 0.5 % Triton X-100. Homogenates were centrifuged for 10 min at 10,000 rpm. Supernatants were mixed with an equivalent volume of SDS buffer (10% SDS in Tris/HCl, pH 8, containing 50% glycerol, 0.1 mM 2-beta-mercaptoetanol and 0.1% Bromophenol blue). The mixture was then denatured for 5 min at 100° C, and aliquots of 10 mul of homogenate were separated on SDS-polyacrylamide slab minigels (15% gradient gels). Separated proteins were transferred to nitrocellulose membranes in the transfer buffer (25 mM Tris-HCl, 192 mM glycine, 0.1% SDS and 20% methanol). Membranes (0.2 mum) were blocked with 3% bovine serum albumin (BSA) dissolved in TBST buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20 pH 8) overnight at 37° C, and then incubated with the primary antibodies at 1:250 dilution in blocking solution for 3 hours: goat polyclonal antibody against bad, rabbit polyclonal antibody against mcl-1, mouse monoclonal antibody against p21, goat polyclonal antibody against phosphorylated retinoblastoma protein (pRB), and mouse monoclonal antibody against retinoblastoma protein (both phosphorylated and dephosphorylated forms) (Rb). The four former antibodies were purchase from Santa Cruz Biotechnologies (Santa Cruz, CA, USA), and the latter from Chemicon (Tamecula, CA, USA). After extensive washing with TBS/Tween 20, the membranes were incubated with a biotin-conjugated anti-mouse, anti-rabbit

(Vector, Laboratories, Burlingame, CA, USA), or anti-goat (Santa Cruz) immunoglobulins for 1 hour at room temperature, and then washed and incubated with the avidin-biotin-peroxidase complex (Vector) at 1:1,000 dilution. The membranes were developed with an enhanced chemiluminescence (ECL) kit, following the procedure described by the manufacturer (Amersham, Buckinghamshire, UK). The staining intensity (optical density) of each band was measured with an automatic image analyser (MIP4, version 4.4, Consulting Image Digital, Barcelona, Spain), in order to compare the expression of each protein between the different groups of prostates. From the individual values (each prostate), the means ± SD for each group (normal, BPH, and PC) were compared by ANOVA and the significance of differences between groups were evaluated by the Fisher and Behrens test.

For enzyme-linked immunoassay (ELISA) the protein concentration of each prostate was calculated by the Bradford method [25], and were diluted to 4.125 mug/mul. Serial dilutions of proteins from each prostate were made and incubated on 96-well multiplates overnight at 4° C. The plates were washed with TBS containing 0.05% Tween 20, blocked with 1% BSA in TBS for 1 hour at room temperature, and incubated with the first antibodies (bad, mcl-1, p21, Rb and pRB) at 1:1,000 dilution for 3 hours, also at room temperature. After another washing, the biotin-conjugated anti-mouse, anti-rabbit (Vector) or anti-goat (Santa Cruz) immunoglobulins were added to each well, incubated for 1 hour at room temperature, and then washed and incubated with the avidin-biotin-peroxidase complex (Vector). The interactions were visualised with 0.05% 2,2 azino di-3-ethylbenzthiazholine sulphonic acid (ABTS) (Sigma, Barcelona, Spain) in 100 mM citrate buffer, pH 5. Optical density values at 405 nm were obtained in a spectrophotometer (Multiskan Bichromatic, Labsystems, Finland). For each prostate the assay was repeated at two days intervals in triplicate? and the average values (expressed as optical density of 4.125 mug/mul of protein) were calculated and represented. From these values, the means ± SD for each prostate group were compared by ANOVA and the significance of differences between groups were evaluated by the Fisher and Behrens test.

For immunohistochemistry, tissues were fixed in a 0.1 M phosphate-buffered 10% formaldehyde solution for 24 hours, dehydrated, and embedded in paraffin. Sections, 5 mum-thick, were processed following the avidin-biotin-peroxidase complex (ABC) method. Microwave antigen retrieval was performed. Briefly, after deparaffinisation, sections were hydrated, incubated for 30 min in 0.3% H_2O_2 in

methanol to inhibit endogenous peroxidase activity and microwave irradiated at 1,300 W in 10 mM citrate buffer at pH 5.8 for 2.5 min, 5 times, letting sections cool down between microwave cycles. After rinsing in TBS buffer, the slides were incubated with normal goat or donkey serum at 1:5 diluted in TBS containing 5% BSA for 30 min to prevent non-specific binding of the first antibody (bad, mcl-1, p21, Rb and pRb). The sections were then incubated overnight at 4° C with primary antibodies, diluted in TBS containing 1% BSA. The primary antibody dilutions found to be optimal for this study were 1:25. The sections were then washed in TBS and incubated with goat anti-mouse or anti-rabbit (Vector) or donkey anti-goat biotinylated immunoglobulins (Santa Cruz). Later, the sections were incubated with avidin-biotin-peroxidase complex (Vector) for 30 min and developed with diaminobenzidine (DAB, Sigma, Barcelona, Spain), using the glucose-oxidase-DAB-nickel intensification method. After this, sections were dehydrated and mounted in DePex (Probus, Badalona, Spain). Care was always taken to develop the sections of the different pathological and non-pathological conditions at exactly the same time in each immunohistochemical reaction.

The specificity of the immunohistochemical procedures was checked using negative and positive control sections. For negative control of immunoreactions, adjacent sections of each type (normal, BPH, and prostatic cancer) were incubated with blocking peptide for bad and pRb antibodies (Santa Cruz). As positive controls, sections of rat skin were incubated with the same antibodies.

A comparative histological quantification of immunolabelling in normal, hyperplastic, and neoplastic prostates was performed for each of the five antibodies. Six histological sections of each region (central, intermediate and peripheral) of each normal prostate were selected at random.

In each section, either (a) the percentage of immunolabelled nuclei were counted in a total of 1,000 nuclei examined per region (for p21, Rb and pRb), or (b) the staining intensity (optical density) per unit surface area of the epithelium and stroma (for bad and mcl-1) were measured with an automatic image analyser (MIP4 version 4.4, Consulting Image Digital, Barcelona, Spain) in 5 light microscopic fields per region. Delimitation of both types of surface areas (epithelium and stroma) was carried out manually using the mouse of the image analyser. The immunostaining intensity of each cell type (basal and columnar

epithelial cells and stromal cells) could not be evaluated because it was not possible to determine the borders of each individual cell. For each positively immunostained section, one negative control section (the next in a series of consecutive sections) was also used, and the optical density of this control section was subtracted from that of the stained section.

Calculations were carried out by two different observers, using the X40 objective. From average values for each prostate, the means ± SD for the normal prostate group were calculated. The same quantitative study was carried out in the hyperplastic and neoplastic prostates, although the number of sections per prostatic region was greater (23 in BPH and 29 in PC), and all these sections were taken from the impaired zone. For the three groups of prostatic specimens, the number of sections examined was determined by successive approaches to obtain the minimum number required to reach the lowest SD. The statistical significance between means was assessed by the Fisher and Behrens test.

RESULTS

Western blot analysis showed a single band for some antigens (p21, bad and mcl-1) at the corresponding molecular weights (20, 20 and 40 kDa respectively). One band, at 110 kDa was found for pRb (phosphorylated form of Rb), and two bands, at 105 kDa (dephosphorylated form) and 110 kDa (phosphorylated form) for Rb antigen, which recognized both forms of retinoblastoma protein (Figure 1). Comparison of optical densities revealed significant differences (p ¾ 0.05) between the three groups of prostates. The highest optical density was found for PC specimens, and the lowest for normal prostates (Table 1).

The ELISA results showed a linear correlation between optical densities and homogenised tissues concentrations (Figure 2). The optical densities obtained for each of the five antibodies (measured in 4.125 mug/mul of protein) agree with the results of Western blot analysis, *i.e.*, optical densities were higher in BPH than in normal prostates, and even higher in PC samples.

No immunoreaction was observed in negative controls incubated with pre-immune serum. Staining of skin sections was always positive.

No or scanty immunoreactivity (< 1% of cells) to p21 was observed in normal prostates (Figure 3). In BPH specimens, positive immunoreaction was identified

in the nuclei of many epithelial cells and some stromal cells (Figure 4). In PC specimens, immunoreaction was similar although the number of epithelial and stromal cell nuclei stained was higher than in BPH (Figure 5).

In normal prostates, Rb staining was positive in isolated epithelial cell nuclei (Figure 6). In BPH specimens, immunoreaction was observed in the nuclei of most epithelial cells and some stromal cells (Figure 7). PC samples showed a similar, although more intense, immunostaining in both epithelium and stroma (Figure 8). The same results were obtained for pRb, although immunoreaction to Rb was more intense than that to pRb in three groups of prostates (Figure 9).

In normal prostates, immunoreaction to bad appeared as a spot in the basal cytoplasm of isolated epithelial cells, and only occasionally in that of stromal cells (Figure 10). In BPH, immunoreaction was similar although it was found in a greater number of epithelial cells (Figure 11). In PC samples, immunoreaction was apparently found in the nuclei of many epithelial cells and occasionally in those of stromal cells (Figure 12).

A weak cytoplasmic immunoreaction to mcl-1 appeared in epithelial cells and some stromal cells (Figure 13) in normal prostates. In BPH (Figure 14) and PC samples (Figure 15), most of epithelial cells showed cytoplasmic immunostaining, and the number of immunostained stromal cells was higher than in normal prostates.

The results obtained from the comparison of optical densities between the three groups of specimens (normal, BPH and PC) for each immunohistochemical staining are shown in Tables 2 and 3.

DISCUSSION

Since PC and BPH are two of the most common diseases in men, there have been numerous reports showing the complexity of growth control in the human prostatic epithelium. The present study was focused on the evaluation of some prognostic markers (p21, Rb, bad and mcl-1 gene products) of the cell cycle progression at G1 phase. Most of previous studies on these four gene products in the prostate refer to PC specimens, whereas normal and hyperplastic prostates have received less attention.

In normal prostates, we failed to find important immunoreactions to any of the four gene products studied here. This agrees with the results of three previous reports that described the absence of immunoreactivity to p21 [7], mcl-1 [24], and bad [20]. Only one study by Shirauski *et al.* [8] has reported positive immunoreaction to p21. No references to Rb in normal prostate have been encountered. Since these four gene products were found in considerable amounts in both BPH and PC specimens, evaluation of these products can be helpful to discriminate between normal prostatic tissues and those undergoing pathological proliferative processes.

The positive immunoreaction to p21 in PC specimens agrees with the results of previous studies [7, 8]. In cancer, the expression of p21 has been significantly associated with a high Gleason score, and suggests an unfavourable prognosis [7]. Since p21 is a repressor of the cell cycle, its marked expression in PC may be interpreted as an attempt by the cell to counteract other factors that promote cell proliferation and that appear largely expressed in PC, such as proliferating cell nuclear antigens, bcl-2, and the mutated form of p53 [7, 9]. This mutation is among the most common genetic alterations in human cancers [26].

P21 expression is normally induced by other suppressor gene, the wild type of p53 [27], but PC specimens express the mutated form of p53 [28, 29], which is unable to activate p21 [3]. It is possible that, in PC, p21 might be induced by other mechanisms including TGF-beta [30], as has been reported in other tumour types [31]. TGF-beta acts as a growth inhibitor of prostatic epithelium [32], and recently, Robson *et al.* [30] reported that this inhibitory effect is regulated by p21 that causes the dephosphorylation of Rb, and then, the arrest of the cell cycle in G1. Increased expression of TGF-beta1 has been reported in PC specimens [33, 34].

The increased expression of both forms (phosphorylated and dephosphorylated) of retinoblastoma protein has been found in the PC specimens studied here and in previous reports [13, 14]. The increase in the phosphorylated form was to be expected, because this form of retinoblastoma promotes cell proliferation [10]. However, the increased expression of the dephosphorylated form of retinoblastoma, which arrests the cell cycle [11], suggests an attempt to hinder cell proliferation.

The ratio, anti-apoptotic (bcl-2, bcl-Xl, mcl-1) to pro-apoptotic (bad, bax, bak, bcl-Xs) proteins of the bcl-2 family seems to control the sensitivity or resistance of many cells to apoptotic stimuli. The presence of anti-apoptotic proteins in PC specimens was to be expected because the proliferation/apoptosis equilibrium is displaced towards cell proliferation. In this report, a marked expression of mcl-1 was observed. Previously, Krajewska *et al.* [24] described that 81% of adenocarcinomas contained mcl-1, and different studies have reported high levels of bcl-2 [35, 36]. However, pro-apoptotic proteins such as bax also seem to be present [24, 36]. To our knowledge, this is the first report that describes the immunolocation of the pro-apoptotic bad protein in PC specimens. The Bcl-21 family has been localized to the mitochondrial membrane, endoplasmic reticulum and nuclear membrane [37]. According to the histochemical images, in PC, immunostaining to bad was probably present in nuclear membranes, whereas in NP and BPH, immunostaining was present in the cytoplasmic membranes. Previously, Kitada *et al.* [20] found marked immunoreaction to this protein in some prostate cells lines such as DU-145 or PC3. The presence of these pro-apoptotic proteins in PC specimens agrees with the results of several authors, who found that the apoptotic index of the prostatic epithelium was higher in PC than in both BPH and normal prostate [38].

In PC specimens, the increased expression of several factors that hinder cell proliferation (such as the p21 and bad gene products studied here) is not sufficient to control tumour growth, because the regulation of proliferation-apoptosis is defective in the majority of advanced prostatic carcinomas. It is possible that additional administration of TGF-beta or p21 would be effective as an attempt to hinder the Rb phosphorylation that induces the entry of cells into the G1 phase of the cell cycle. In this way, prospective clinical investigation will be necessary in order to find the best agent to restore the proliferation-apoptosis equilibrium.

In BPH specimens, immunoexpression of the four gene products studied (p21, Rb, mcl-1 and bad) was higher than in normal prostates, although their expression was not as elevated as in PC. Previous studies have shown similar findings for many other protein gene products involved in the cell cycle including IFN-gamma [30], myc [30, 39], mutated p53 [29, 30], cell proliferation nuclear antigen ki-67 [41], estrogen receptors (ERalpha, ERbeta) [41, 42], and ERK [43]. The levels of other proteins, such as EGF [44], TGF-alpha [45], IL-2 [36, 45], and bcl-2 [24] were found to be as high in BPH as in PC. These findings suggest that

overexpression of all these gene products does not necessarily mean a malignant transformation of prostatic tissue, because these high levels are also associated, with a benign prostatic cell proliferation. However, other factors that showed higher expression in PC than in both BPH and normal prostates, such as TGF-beta1 [34, 46], bax [24], and JNK [47], seem to be more indicative of neoplastic transformation of prostatic cells.

CONCLUSION

Acknowledgements. This work was supported by grants from the Fondo de Investigaciones sanitarias (98/028), and Sociedad Madrileña de Urología.

References

1. Lu S, Liu M, Epner D E, Tsai S Y, Tsai M J. 1999. Androgen regulation of the cyclin-dependent kinase inhibitor p21 gene through an androgen response element in the proximal promoter. *Mol. Endocrinol.* 13: 376.

2. Serrano M, Hannon G J, Beach D. 1993. A new regulatory motif in cell cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 366: 704.

3. El-Deiry W S, Tokino T, Waldman T, Oliner J D, Velculescu V E, Burrell M, Hill D E, Healy R, Rees J L, Hamilton S R, Kinzler K W, Vogelstein B. 1995. Topological control of p21(WAF1/CIP1) expression in normal and neoplastic tissues. *Cancer Res.* 55: 2910.

4. Harper J W, Adami G R, Wei N, Keyomarski K, Elledge S J. 1993. The p21 Cdk-interacting protein Cip 1 is a potent inhibitor of G1 cyclin-dependent kinases. *Cell* 75: 805.

5. Sherr C J. 1994. G1 phase progression: cycling on cue. (Review) *Cell* 79: 551.

6. Waga S, Hannon G J, Beach D, Stillman B. 1994. The p21 inhibitor of cyclin dependent kinases controls DNA replication by interaction with PCNA. *Nature* 369: 574.

7. Aaltomaa S, Lipponen P, Eskelinen M, Ala-Opas M, Kosma V M. 1999. Prognostic value and expression of p21 (WAF1/Cip 1) protein in prostate cancer. *Prostate* 39: 8.

8. Shiraishi T, Watanabe M, Muneyuki T, Nakayama T, Morita J, Ito H, Kotake T, Yatani R. 1998. A clinicopathological study of p53, p21 (WAF1/CIP1) and cyclin D1 expression in human prostate cancers. *Urol. Int.* 61: 90.

9. Baretton G B, Klenk U, Diebold J, Schmeller N, Löhrs U. 1999. Proliferation- and apoptosis-associated factors in advanced prostatic carcinomas before and after androgen deprivation therapy: prognostic significance of p21/WAF1/CIP1 expression. *Br. J. Cancer* 80: 546.

10. Dowdy S F, Hinds P W, Louie K, Reed S I, Arnold A, Weinberg R A. 1993. Physical interaction of the retinoblastoma protein with human D cyclins. *Cell* 73: 499.

11. Chen P L, Scully P, Shew J Y, Wang J Y, Lee W H. 1989. Phosphorylation of the retinoblastoma gene product is modulated during the cell cycle and cellular differentiation. *Cell* 58: 1193.

12. Blagosklonny M V, Prabhu N S, El-Deiry W S. 1997. Defects in p21WAF1/CIP 1, Rb and c-myc signaling in phorbol ester-resistant cancer cells. *Cancer Res.* 57: 320.

13. Phillips S M , Barton C M, Lee S J, Morton D G, Wallace D M, Lemoine N R, Neoptolemus J P. 1994. Loss of the retinoblastoma susceptibility gene (RB1) is a frequent and early event in prostatic tumorigenesis. *Br. J. Cancer* 70: 1252.

14. Theodorescu D, Broder S R, Boyd J C, Mills SE, Frierson H F. 1997. p53, bcl-2 and retinoblastoma proteins as long-term prognostic markers in localized carcinoma of the prostate. *J. Urol.* 158: 131.

15. Oltvai Z N, Milliman C L, Korsmeyer S J. 1993. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* 74: 609.

16. Yin X M, Oltvai Z N, Korsmeyer S J. 1994. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature* 369: 321.

17. Boyd J M, Gallo G J, Elangovan B, Houghton A B, Malstrom S, Avery B J, Ebb R G, Subramaniam T, Chittenden T, Lutz R J. 1995. Bik, a novel death-inducing protein shares a distinct sequence motif with Bcl-2 family proteins and interacts with viral and cellular survival proteins. *Oncogene* 11: 1921.

18. Datta S R, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg M E. 1997. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell* 91: 231.

19. Yang T, Kozopas K M, Craig R W. 1995. The intracellular distribution and pattern of expression of Mcl-1 overlap with, but are not identical to, those of Bcl-2. *J. Cell. Biol.* 128: 1173.

20. Kitada S, Krajewska M, Zhang X, Scudiero D, Zapata J M, Wang H G, Shabaik A, Tudor G, Krajewski S, Myers T G, Johnson G S, Sausville E A, Reed J C. 1998. Expression and location of pro-apoptotic Bcl-2 family protein BAD in normal human tissues and tumor cell lines. *Am. J. Pathol.* 152: 51.

21. Kozopas K M, Yang T, Buchan H L, Zhou P, Craig R W. 1993. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. *Proc. Natl. Acad Sci. USA* 90: 3516.

22. Moulding D A, Quayle J A, Hart C A, Edwards S W. 1998. Mcl-1 expression in human neutrophils: regulation by cytokines and correlation with cell survival. *Blood* 92: 2495.

23. Krajewki S, Bodrug S, Krajewska M, Shabaik A, Gascoyne R, Berean K, Reed J C. 1995. Immunohistochemical analysis of Mcl-1 protein in human tissues. Differential regulation of Mcl-1 and Bcl-2 protein production suggests a unique role for Mcl-1 in control of programmed cell death *in vivo. Am. J. Pathol.* 146: 1309.

24. Krajewska M, Krajewski S, Epstein J I, Shabaik A, Sauvageot J, Song K, Kitada S, Reed J C. 1996. Immunohistochemical analysis of Bcl-2, bax, Bcl-X and Mcl-1 expression in prostate cancers. *Am. J. Pathol.* 148: 1567.

25. Bradford M M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* 72: 248.

26. Levine A J, Momand J, Finlay C A. 1991. The p53 tumour suppressor gene. (Review) *Nature* 351: 453.

27. Macleod K F, Sherry N, Hannon G, Beach D, Tokino T, Kinzler K, Vogelstein B, Jacks T. 1995. p53-dependent and independent expression of p21 during cell growth, differentiation and DNA damage. *Genes Dev.* 9: 935.

28. Mellon K, Thompson S, Charlton R G, Marsh C, Robinson M, Lane D P, Harris A L, Horne C H, Neal D E. 1992. p-53, c-erbB2 and the epidermal growth factor receptor in the benign and malignant prostate. *J. Urol.* 147: 496.

29. Royuela M, de Miguel M P, Ruiz A, Fraile B, Arenas M I, Romo E, Paniagua R. 2000. Interferon-gamma and its functional receptors overexpression in benign prostatic hyperplasia and prostatic carcinoma: parallelism with c-myc and p53 expression. *Eur. Cytokine Netw.* 11: 119.

30. Robson C N, Gnanapragasam V, Byrne R L, Collins A T, Neal D E. 1999. Transforming growth factor-beta1 up-regulates p15, p21 and p27 and blocks cell cycling in G1 in human prostate epithelium. *J. Endocrinol.* 160: 257.

31. Datto M B, Li Y, Panus J F, Howe DJ, Xiong Y, Wang X F. 1995. Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc. Natl. Acad. Sci. USA* 92: 5545.

32. Story M T. 1991. Polypeptide modulators of prostatic growth and development. (Review) *Cancer Surv.* 11: 123.

33. Royuela M, de Miguel M P, Bethencourt F R, Sánchez-Chapado M, Fraile B, Paniagua R. 1998. Transforming growth factor beta1 and its receptor types I and II. Comparison in human normal prostate, benign prostatic hyperplasia, and prostatic carcinoma. *Growth Factors* 16: 101.

34. Truong L, Kadmon D, McCune B, Flanders K C, Scardino P T, Thompson T C. 1993. Association of transforming growth factor-beta1 with prostate cancer: an immunohistochemical study. *Hum. Pathol.* 24: 4.

35. McDonnell T J, Troncoso P, Brisbay S M, Logothetis C, Chung L W, Hsieh J T, Tu S M, Campbell M L. 1992. Expression of the proto-oncogene Bcl-2 in the prostate

and its association with emergence of androgen-independent prostate cancer. *Cancer Res.* 52: 6940.

36. Royuela M, de Miguel M P, Bethencourt F R, Fraile B, Arenas M I, Paniagua R. 2000. IL-2, its receptors, and Bcl-2 and bax genes in normal, hyperplastic and carcinomatous human prostates: immunohistochemical comparative analysis. *Growth Factors* 18: 135.

37. De Jong D, Prins F A, Masson D Y, Reed J C, van Ommen G B, Kluin P M. 1994. Subcellular localization of the Bcl-2 protein in malignant and normal lymphoid cells. *Cancer Res.* 54: 256.

38. Aihara M, Scardino P T, Truong L D, Wheeler T M, Goad J R, Yang G, Thompson T C. 1995. The frequency of apoptosis correlates with the prognosis of Gleason grade 3 adenocarcinoma of the prostate. *Cancer* 75: 522.

39. Marcu K B, Bossone S A, Patel A J. 1992. myc function and regulation (Review). *Annu. Rev. Biochem.* 61: 809.

40. Gallee M P, Visser-de Jong E, ten-Kate F J, Schroeder F H, Van der Kwast T H. 1989. Monoclonal antibody Ki-67 defined growth graction in benign prostatic hyperplasia and prostatic cancer. *J. Urol.* 142: 1342.

41. Royuela M, de Miguel M P, Bethencourt F R, Sánchez-Chapado M, Fraile B, Arenas M I, Paniagua R. 2001. Estrogen receptors alpha and beta in the normal, hyperplastic and carcinomatous human prostate. *J. Endocrinol.* 168: 447.

42. Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. 1999. Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. *Am. J. Pathol.* 155: 641.

43. Price D T, Rocca G D, Guo C, Ballo M S, Schwinn K, Luttrell L M. 1999. Activation of extracellular signal-regulated kinase in human prostate cancer. *J. Urol.* 162: 1537.

44. Habib F.K. 1990. Peptide growth factors: a new frontier in prostate cancer. (Review) *Prog. Clin. Biol. Res.* 357: 107.

45. Wilding G, Valverius E, Knabbe C, Gelmann E P. 1989. Role of transforming growth factor-alpha in human prostate cancer cell growth. *Prostate* 15: 1.

46. Triest J A, Grignon D J, Cher M L, Kocheril S V, Montecillo E J, Talati B, Tekyi-Mensah S, Pontes J E, Hillman G G. 1998. Systemic interleukin-2 therapy for human prostate tumors in a nude mouse model. *Clin. Cancer Res.* 4: 2009.

47. Magi-Galluzzi C, Mishra R, Fiorentino M, Montironi R, Yao H, Capodieci P, Wishnow K, Kaplan I, Stork P J, Loda M. 1997. Mitogen-activated protein kinase phosphatase 1 is overexpressed in prostate cancers and in inversely related to apoptosis. *Lab. Invest.* 76: 37.

Figures

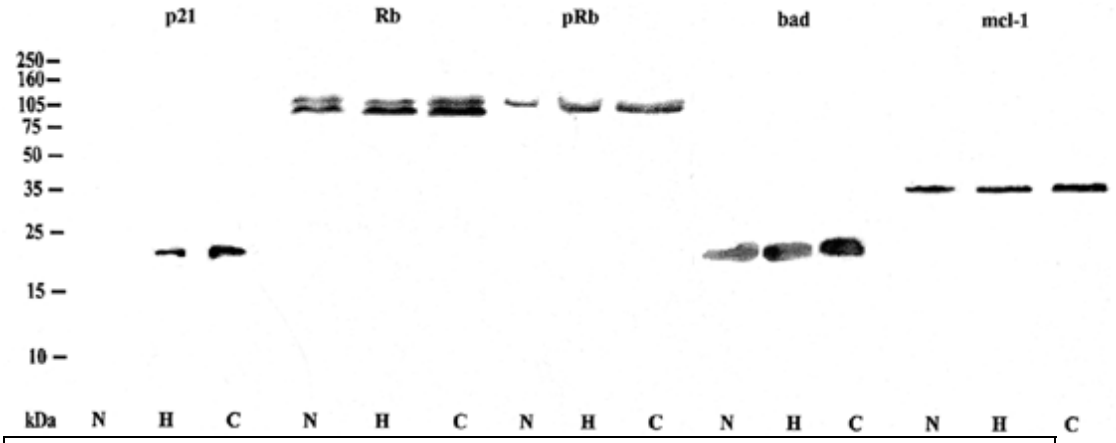


Figure 1
Western blot analysis of p21, Rb (phosphorylated and dephosphorylated forms), pRb (phosphorylated form), bad and mcl-1 gene products after 15% polyacrylamide gel electrophoresis. N: normal prostate. H: benign prostatic hyperplasia. C: prostatic carcinoma. Molecular weights are shown on the left. Of the two bands found for Rb, the upper band corresponds to the phosphorylated forms, and the lower band to the dephosphorylated form.

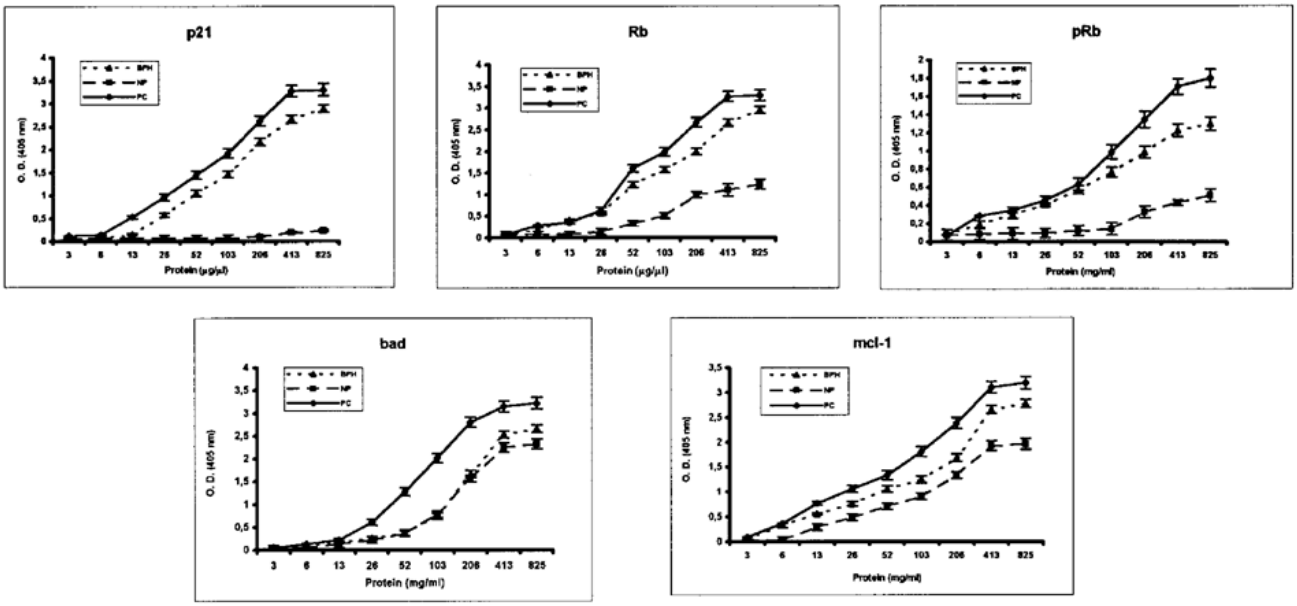
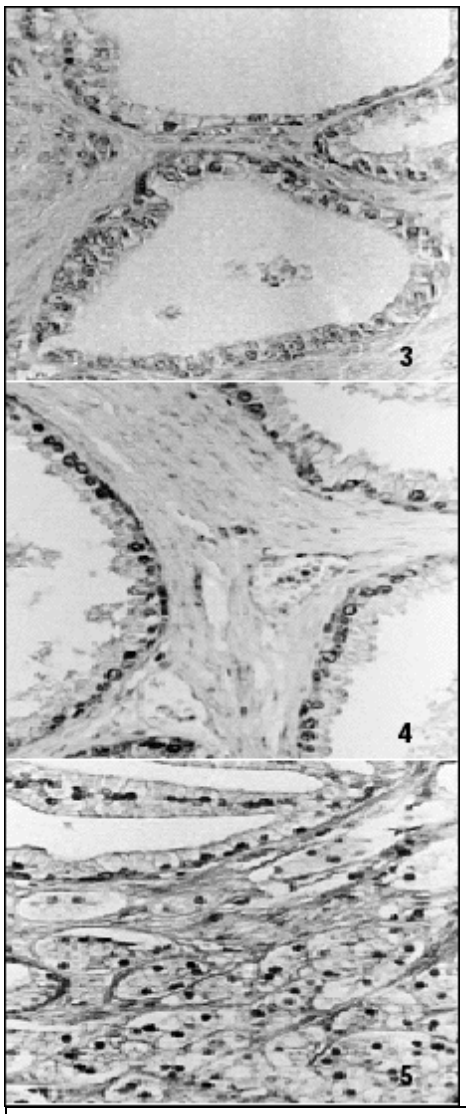
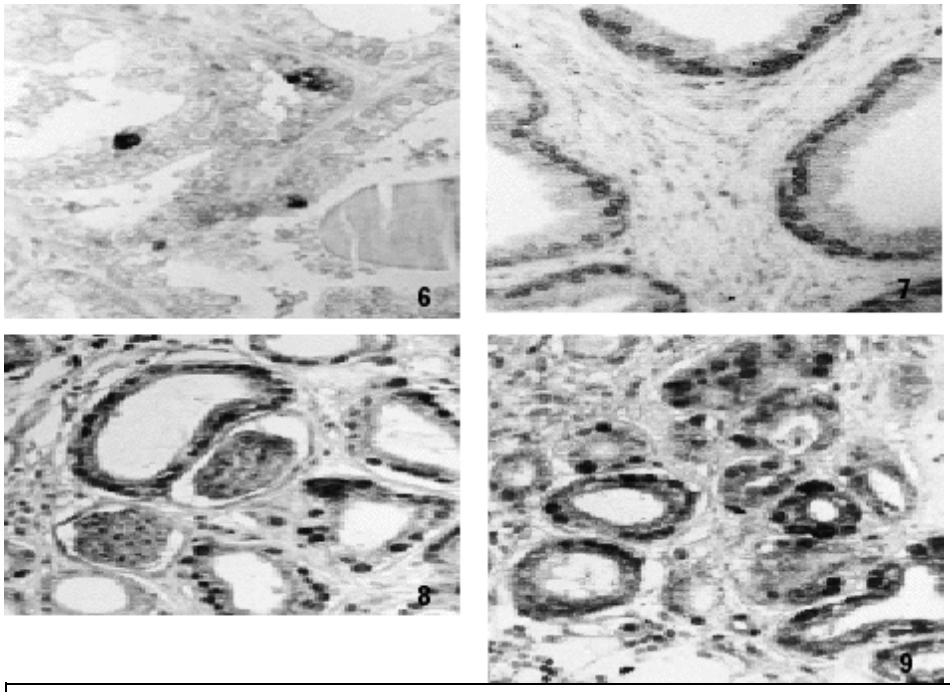


Figure 2

ELISA determinations of p21, Rb (phosphorylated and dephosphorylated forms), pRb (phosphorylated form), bad and mcl-1 gene products in normal prostates (NP), benign prostatic hyperplasia (BPH), and prostatic carcinoma (PC). Antibody bindings were followed at 405 nm, using peroxidase-conjugated anti-mouse (p21, Rb), anti-goat (bd) or anti-rabbit (mcl-1) immunoglobulins. The mean values and SD for each group have been calculated from all samples corresponding to the group. O.D. = optical density.

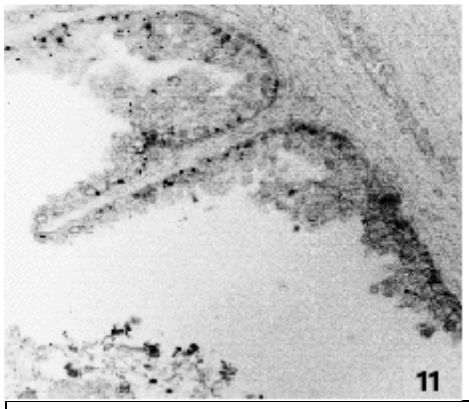


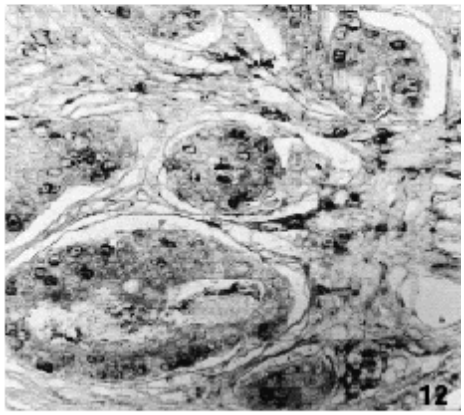
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Immunoreaction to p21 in human prostates following the ABC method. Figure 3: normal prostate showing a very weak immunoreaction. Figure 4: benign prostatic hyperplasia. The nuclei of many epithelial cells and some stromal cells are positively stained. Figure 5: prostatic carcinoma. Immunoreaction is similar to that observed in benign prostatic hyperplasia, although the number of stained cells is higher. **x** 450.



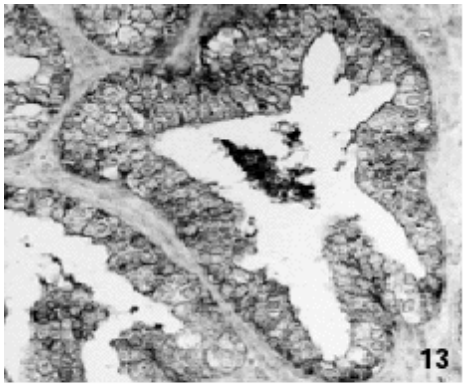
Figures 6-9
Immunostaining to Rb (Figures 6-8) and pRB (Figure 9) in human prostates following the ABC method. Figure 6: normal prostate showing only a few immunolabelled epithelial cell nuclei. Figure 7: benign prostatic hyperplasia. The nuclei of many epithelial cells and some stromal cells appeared immunolabelled. Figure 8: prostatic carcinoma. Immunoreaction is similar to that observed in benign prostatic hyperplasia, although the number of stained cells is higher. Figure 9: immunostaining to pRb in prostatic carcinoma is similar to that observed for Rb, although the number of stained cells is lower. **x** 450.

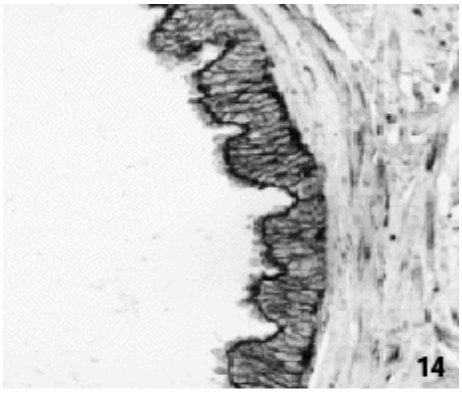


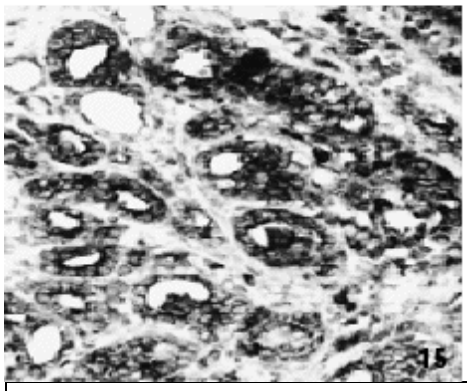




Figures 10-12
Immunostaining to bad in human prostates following the ABC method. Figure 10: normal prostate showing only a few immunolabelled epithelial cells. Immunolabelling appears as a cytoplasmic spot. Figure 11: benign prostatic hyperplasia. Immunolabelling is similar but appeared in many epithelial cells. Figure 12: prostatic carcinoma. Cytoplasmic immunostaining of epithelial cells is more intense than in benign prostatic hyperplasia. **x** 450.







Figures 13-15
Immunostaining to mcl-1 in human prostates following the ABC method. Figure 13: normal prostate showing scanty immunostaining. Figure 14: benign prostatic hyperplasia. The cytoplasmic of many epithelial cells and some stromal cells appeared immunostained. Figure 15: prostatic carcinoma. Cytoplasmic immunostaining of epithelial cells is more intense than in benign prostatic hyperplasia. **x** 450.

Table 1
Comparison of immunostaining intensities (measured as average optical density ± SD) in Western blot analyses of normal prostates, benign prostatic hyperplasia, and prostatic carcinoma

Prostates	Normal prostate	Benign prostatic hyperplasia	Prostatic carcinoma
p-21	0	71.76 ± 6.9[a]	90.58 ± 8.2[b]
Rb			
110 kDa (phosphorylated)	29.41 ± 1.9[a]	35.6 ± 4.1[b]	47.21 ± 3.2[c]
105 kDa (dephosphorylated)	43.52 ± 4.9[c]	62.35[e]	76.07[f]
pRb	27.25 ± 4.2[a]	39.44 ± 4.6[b]	45.09 ± 3.7[c]
Bad	38.8 ± 3.5[b]	57.6 ± 4.5[d]	64.7 ± 2.6[e]
Mcl-1	70.3 ± 6.8[c]	79.6 ± 7.5[f]	82.74 ± 4.3[g]

For each protein, values with a different superscript letter differ significantly ($P \leq 0.05$) between them. The mean values and SD for each group have been calculated from all samples corresponding to the group.

Table 2
Comparison of the percentage of immunolabelled nuclei of the epithelium and stroma of the prostate in normal prostates, benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma

Prostates	P21	Rb	pRb
Normal			
Epithelium	0.65 ± 0.7[a]	4.66 ± 1.36[a]	1.35 ± 0.91[a]
Stroma	0.75 ± 0.7[a]	0.23 ± 0.21[b]	0.14 ± 0.27[a]
BPH			
Epithelium	66.83 ± 5.4[b]	64.37 ± 8.19[c]	15.78 ± 9.65[b]
Stroma	4.78 ± 2.94[c]	4.17 ± 1.50[a]	2.05 ± 0.98[a]
Cancer			
Epithelium	84.82 ± 6.41[d]	80.15 ± 12.32[d]	21.24 ± 11.67[c]
Stroma	13.32 ± 1.51[c]	28.30 ± 1.81[c]	6.9 ± 2.45[d]

For each antigen (column), values with a different superscript letter differ significantly ($p \leq 0.05$). The mean values and S.D. for each group have been calculated from all samples corresponding to the group.

Table 3
Comparison of immunostaining intensities (measured as optical densities) of the epithelium and stroma of the prostate in normal prostates, benign prostatic hyperplasia (BPH) and prostatic adenocarcinoma

Prostates	Bad	Mcl-1
Normal		
Epithelium	19.12 ± 2.10[a]	20.10 ± 2.23[a]
Stroma	2.94 ± 1.75[b]	6.31 ± 0.89[b]
BPH		
Epithelium	25.59 ± 3.25[c]	24.50 ± 2.30[c]
Stroma	3.13 ± 2.1[b]	6.17 ± 1.50[b]
Cancer		
Epithelium	37.18 ± 4.56[d]	29.41 ± 3.26[d]
Stroma	5.01 ± 2.23[e]	15.67 ± 2.1[e]

For each antigen (column), values with a different superscript letter differ significantly ($p \leq 0.05$). The mean values and S.D. for each group have been calculated from all samples corresponding to the group.

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Recombinant human interleukin-1 beta: new possibilities for the prophylaxis and correction of toxic myelodepression in patients with malignant tumors I. Phase I-II clinical trials of recombinant human interleukin-1 beta as a leukopoiesis stimulator in cancer patients receiving combination chemotherapy

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human recombinant interleukin-1 beta, myelopoiesis, myelodepression, cancer chemotherapy.

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