Ki-67 expression in early prostate cancer and associated pathological lesions

M R Feneley, M P A Young, C Chinyama, R S Kirby, M C Parkinson

Abstract

***Aim*—To assess cell proliferation in early prostate cancer and associated pathological lesions.**

***Methods*—Using the Ki-67 antibody, the cell proliferation index was measured in early stage prostatic carcinoma in 37 incidental tumours diagnosed at transurethral prostatectomy (TURP) and in 20 low volume cancers treated by radical prostatectomy. Proliferation indexes have also been measured in areas of normal peripheral zone, transition zone hyperplasia, atrophic appearing lobules, and high grade prostatic intraepithelial neoplasia in the radical prostatectomy cases.**

***Results*—In the TURP series the proliferation index correlated with grade and stage. Logistic regression analysis, however, showed that Gleason grade was the most reliable predictor of biopsy proven residual disease and clinical progression. In the radical series transition zone carcinoma the proliferation index was half that of peripheral zone carcinoma. The atrophic lobules also showed a high proliferation index of the same order as seen in the peripheral zone carcinoma. Normal peripheral zone showed the lowest proliferation index and in hyperplastic transition zone it was also less than the other areas.**

***Conclusions*—There is only limited support for the correlation of proliferation index with grade in early stage prostatic carcinoma. The findings do not suggest that proliferation index adds to the prognostic information given by grade and stage in pT1 disease. The significant difference in proliferation index in transition zone and peripheral zone carcinomas supports the morphological distinction of these tumour types and is consistent with differences in biological behaviour. The high proliferation index in lobules considered morphologically atrophic is reminiscent of previous observations in which carcinoma was spatially associated with atrophy.**

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Cell proliferation is a fundamental aspect in a number of prostatic diseases ranging from hyperplasia to neoplasia and can be studied using antibodies directed against nuclear antigens expressed in certain phases of the proliferation cycle, such as Ki-67.[1] Such techniques are more objective and sensitive than previous methods based on mitotic counts.[2–5] In particular, cellular proliferation can be studied using the immunocytochemical marker directed against the nuclear antigen Ki-67.[5] This is expressed by dividing cells in all phases of the cell cycle other than G_0. The polyclonal antibody directed against Ki-67 was first used in frozen tissue where, in the prostate, its reactivity was shown to correlate with that of proliferating cell nuclear antigen (PCNA), another antigen seen in cells in cycle.[6] With heat enhancement for antigen retrieval, it is possible to use the polyclonal antibody directed against Ki-67 in formalin fixed, paraffin wax sections[7]; the monoclonal antibody MIB-1 has since become available[8] and also recognises this antigen in paraffin wax sections. By determining the proportion of positively staining nuclei, a quantitative estimate of the tumour proliferation index can be given. These immunocytochemical techniques correlate well with other gold standard methods of determining cell proliferation, such as the incorporation of labelled thymidine, or the non-radioactive analogue bromodeoxyuridine; such studies have been undertaken in the prostate.[9]

The prognosis for patients with early stage prostate cancer is notoriously unpredictable. The major aim of this study was to assess the value of the Ki-67 proliferation index in such tumours using two approaches. The proliferation index was assessed in transurethral resection (TUR) specimens of stage T1 disease[10] and low volume disease in radical prostatectomy specimens to determine whether it correlates with the established prognostic features (grade and stage). In the TUR group the ability of the proliferation index to predict subsequent residual biopsy proven carcinoma and progression of malignancy was tested. Furthermore, on the whole mount sections from radical prostatectomy specimens the proliferation index of tumours arising predominantly in the peripheral and transition zones, respectively, was assessed, together with that of normal acini, hyperplasia, atrophy, and high grade prostatic intraepithelial neoplasia (PIN).

Methods

This investigation is comprised of two studies in which there are minor differences in methodology but similar aims, prompting a single publication.

Table 1 Correlation between Ki-67 proliferation index, pathological findings and follow up data

Parameter	n	Ki-67 proliferation index (%) Mean (SE)	Range	Mann-Whitney p value
Grade				
Well differentiated (Gleason sum score < 4)	17	2.29 (0.54)	0–6	Well *v* moderate p = 0.0001
Moderately differentiated (Gleason sum scores 5 and 6)	13	8.15 (1.13)	3–18	Moderate *v* poor p = 0.005
Poorly differentiated (Gleason sum score > 6)	7	16.86 (3.61)	10–37	Well *v* poor p = 0.0001
High grade PIN				
Absent	30	5.4 (0.87)	0–18	p = 0.01
Present	7	14.4 (4.31)	5–37	
Tissue fragments involved (%)				
< 5%	17	3.88 (0.94)	0–12	p = 0.007
> 5%	20	9.85 (1.87)	0–37	
Tumour stage				
pT1a	25	4.8 (0.81)	0–13	p = 0.01
pT1b	12	11.9 (2.87)	0–37	
Follow up biopsy				
Benign	19	4.2 (0.88)	0–13	p = 0.01
Carcinoma	18	10.2 (2.07)	0–37	
Status at follow up				
No progression	18	4.3 (0.90)	0–13	p = 0.1
Progression	13	9.2 (2.77)	0–37	

pT1 CARCINOMA AT TRANSURETHRAL PROSTATECTOMY (TURP)

TURP specimens from 37 patients with previous pT1 prostatic carcinoma undergoing follow up at St Bartholomew's Hospital were studied. The patients were between 61 and 90 years of age (median 72 years) and all had undergone TURP for clinically benign glandular enlargement.

The entire specimen from each patient had been processed routinely to paraffin wax; 3 μm sections were stained with haematoxylin and eosin and reviewed by one pathologist. Tumours were staged according to the proportion of fragments involved and grade. Well differentiated carcinoma in less than 5% of the fragments was defined as stage pT1a; more extensive or less well differentiated carcinoma was defined as pT1b. The presence or absence of high grade PIN was also noted.

Adjacent sections to those showing the abnormalities were stained immunocytochemically with a polyclonal antibody directed against the Ki67 antigen (Dako, High Wycombe, UK; code A047) using the avidin-biotin peroxidase technique with diaminobenzidine as the chromogen and heat enhancement antigen retrieval.[7] Within areas of adenocarcinoma, the cells which had entered the proliferation cycle (identified by positive nuclear staining) were counted within a total of 200 tumour cells. Thus the proliferation index estimated was expressed as the number of positive nuclei per 100 tumour cells.

Patients were followed up for one to eight years (median 40 months) with serial serum prostate specific antigen (PSA) measurements (Hybritech) and were treated until there was evidence of clinical progression. The presence of potentially clinically significant residual malignancy was evaluated initially by systematic quadrant ultrasound guided biopsy specimens taken no less than three months after surgery.

The Ki-67 proliferation index was compared with pathological findings in the TURP specimen including tumour stage, grade and presence of PIN. These were related to residual malignancy on biopsy and subsequent disease progression in untreated patients and then evaluated against PSA slope (% increase per year)* or the development of metastatic disease on bone scan.

RADICAL PROSTATECTOMY SPECIMENS

From the histopathology files of UCL Hospitals NHS Trust 20 radical prostatectomy specimens were selected in which cancer

Table 2 Individual case mean (SE) MIB-1 proliferation indexes(%)

Case number	Age (years)	Tumour volume (ml)	Grade (Gleason)	Normal peripheral zone Total (n = 16)	Basal (n = 11)	Lumen (n = 11)	Hyperplasia of the transition zone Total (n =)	Basal (n = 9)	Lumen (n = 9)	Peripheral zone atrophy (n = 14)
1	66	0.39	2 + 3	0.85						
2	54	0.8	3 + 4	0.93						4.73
3	47	2.5	3 + 4	0.20						4.80
4	66	3.9	3 + 4	0.10						2.60
5	65	4.0	4 + 3	0.47			0.6			4.20
6	74	0.5	3 + 3							
7	54	0.7	3 + 3	1.81	1.30	0.68				2.23
8	63	0.2	3 + 3				1.22	0.59	0.63	
9	69	1.2	3 + 4	0.49	0.35	0.49	2.71	1.76	0.95	
10	69	2.1	3 + 3				0.99	0.67	0.32	5.40
11	65	0.5	2 + 3	0.78	0.28	0.50	1.09	0.85	0.24	3.40
12	62	3.0	2 + 2	0.62	0.35	0.27	1.31	0.74	0.57	
13	60	1.2	3 + 4	0.92	0.47	0.45				5.20
14	51	2.0	3 + 3	1.81	0.95	0.86				4.00
15	58	2.1	3 + 3	0.95	0.46	0.24				9.33
16	57	1.0	3 + 5							
17	57	3.0	2 + 3	0.41	0.37	0.05	1.81	1.52	0.29	1.90
18	73	0.5	2 + 2	0.43	0.37	0.06	2.13	1.32	0.81	5.13
19	69	0.4	4 + 4	0.48	0.23	0.13	1.36	0.81	0.55	6.00
20	57	2.4	3 + 2	0.90	0.70	0.2				
Mean (SE)				0.77 (0.08)	0.53 (0.08)	0.34 (0.05)	1.50 (0.12)	1.02 (0.10)	0.55 (0.06)	4.80 (0.36)

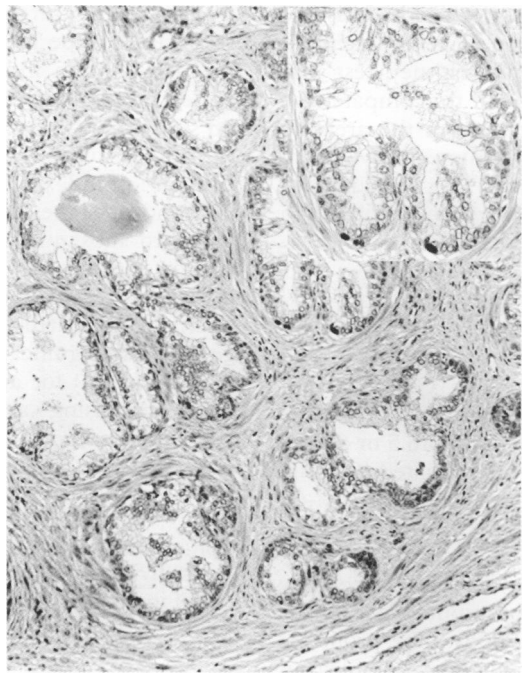

Figure 1 Normal peripheral zone: occasional basal cell nuclei showing MIB-1 positivity (immunoperoxidase).

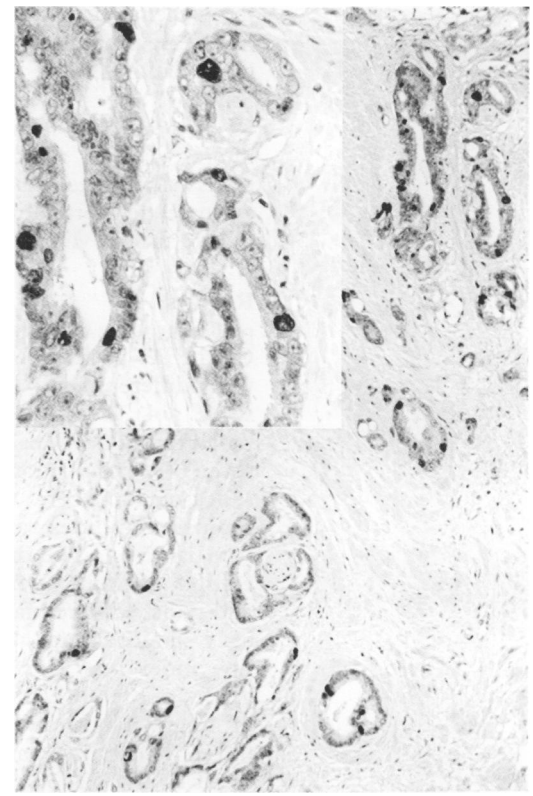

Figure 2 Invasive adenocarcinoma: numerous nuclei showing MIB-1 positivity (immunoperoxidase).

volume did not exceed 4.0 ml and in which tumour site of origin (transition zone, peripheral zone or both) could be assigned according to the location of the largest tumour area. All the glands were examined according to a standard protocol[12] based on the technique described by Epstein.[13] This method produces whole mount sections at standard intervals throughout the gland.

On the haematoxylin and eosin stained sections, areas which showed normal peripheral zone acini, peripheral zone atrophy, hyperplasia of the transition zone, high grade PIN, peripheral zone adenocarcinoma, and transition zone adenocarcinoma were identified and marked. It was not always possible to identify all of the areas of interest in each case. Adjacent whole mount sections were cut and mounted on coated slides (vectabond; Vector Laboratories, Peterborough, UK). After heat pre-treatment by pressure cooking, the sections were stained with the monoclonal antibody MIB-1 (Immunotech, purchased from The Binding Site, Birmingham, UK) using the avidin-biotin peroxidase technique with diaminobenzidine as the chromogen.[14]

In each area of interest counting was confined to sites with the greatest density of positive cells. This location was marked for reproducibility assessment by a second observer. In the normal peripheral zone, hyperplasia of the transition zone and areas of high grade PIN, positively labelled basal cells and luminal cells were counted separately in cases 7–20, but unlabelled basal and luminal nuclei were summated. A minimum of three counts of 500 cells were carried out for each area. The proliferation index was expressed as positive cells per 100 cells counted.

PIN			*Transition zone adenocarcinoma (n = 9)*	*Peripheral zone adenocarcinoma (n = 18)*
Total (n = 14)	*Basal (n = 8)*	*Lumen (n = 7)*		
1.49				3.10
3.25				11.60
				1.07
3.23				3.93
1.15			3.27	4.25
				5.95
1.13				4.01
			0.40	
2.21	0.67	1.73		6.76
3.87		3.87	5.30	4.95
2.93	0.33	2.20		7.20
0.93	0.13	0.80	1.73	7.53
				1.80
			2.67	5.33
			2.67	
1.60	0.40	1.20		3.70
1.40	0.07	1.33		5.20
2.40	0.04	2.00	4.10	9.00
2.37	0.23	2.13	3.60	4.24
9.70				6.50
2.58 (0.29)	0.31 (0.05)	1.93 (0.21)	2.79 (0.28)	5.25 (0.34)

REPRODUCIBILITY OF OBSERVATION

Fifteen cases were counted by a second pathologist to assess reproducibility of observation. No significant statistical difference was found between the values obtained by the two observers. Therefore, the results have been pooled to obtain mean proliferation indexes for each area from each case. Repeat counts were not performed for the TURP series as the proliferation indexes from both series are all of the same order.

STATISTICAL ANALYSIS

In the pT1 TUR series the logistic regression test was used to evaluate the predictive value of age, grade, percentage of fragments of prostate involved, and Ki-67 proliferation index against

*PSA slope was defined as the annual incremental increase in serum PSA expressed as a percentage with a cut-off at 20% increase per year.[11]

the presence of biopsy proven residual disease or disease progression in patients with incidental carcinoma. The Mann-Whitney test and Student's *t* test were used to compare the Ki-67 proliferation indexes from the areas of interest in the radical prostatectomy specimens. The Spearman rank test was used to evaluate the correlation between proliferation index, tumour volume and grade.

Results

pT1 CARCINOMA AT TURP

Of the 37 patients evaluated, 17 (46%) had well differentiated tumours (Gleason sum score < 4), 13 (35%) had moderately differentiated tumours (Gleason sum score 5 or 6) and seven (19%) had poorly differentiated tumours (Gleason sum score > 6) (table 1). The Ki-67 proliferation index varied between 0 and 37% (median 5%). The difference in mean proliferation index in patients with well, moderately and poorly differentiated tumours was statistically significant ($p \leq 0.005$). The proliferation index was also correlated with Gleason grade ($p < 0.001$, rho = 0.79) and percentage of fragments involved ($p < 0.001$, rho = 0.63). Twenty five patients (68%) were staged as pT1a and 12 (32%) as pT1b; the proliferation index was significantly greater in patients with pT1b disease than in those with pT1a disease ($p = 0.01$). Seven tumours were associated with high grade PIN; of these cases, the proliferation index was significantly greater than those without high grade PIN ($p = 0.01$).

All patients subsequently underwent ultrasound guided prostatic biopsy and residual carcinoma was confirmed histologically in 18 (49%). In these patients the proliferation index was significantly greater in the initial TUR tumour than in the remaining 19 (51%) patients who had negative biopsy specimens ($p = 0.01$). Grade, percentage of fragments involved, stage, Ki-67 proliferation index, the presence of high grade PIN, and age were analysed as variables. The most reliable predictor of biopsy proven residual malignancy was Gleason sum score (odds ratio 2.53) with an accuracy of 73% ($p < 0.0001$). After including grade, no other variable, including proliferation index, was significant ($p > 0.05$).

Follow up with serial PSA concentrations was available in 31 untreated patients. Of these, 18 (58%) had no clinical evidence of disease progression and a PSA slope less than 20% per year. Thirteen (42%) had a postoperative PSA slope greater than 20% per year. The proliferation index was not significantly greater in tumours from those patients who had subsequent progression ($p = 0.1$). Analysing grade, tumour volume, clinical stage, proliferation index, the presence of high grade PIN, and age, the variable most significantly related to subsequent progression was higher Gleason score (odds ratio 2.71, $p = 0.0002$), predicting progression appropriately in 77% of patients. After correcting for grade, increasing age was also significant (for grade, odds ratio 2.83, $p = 0.01$; for age, odds ratio 1.33, $p = 0.04$), increasing accuracy to 87%. After including grade and age, other variables were not statistically significant.

RADICAL PROSTATECTOMY SPECIMENS

The individual proliferation indexes from each area of interest are shown in table 2 and related illustrations in figs 1–3.

BASAL CELL MARKING

Despite the greater proliferation index seen in hyperplasia of the transition zone, it can be seen that in both normal peripheral zone, and hyperplasia of the transition zone, basal cells contribute the same proportion of cells showing labelling (68% cells in cycle in both areas) (fig 4). In acini showing PIN, the proportion of basal cells with positive labelling is noticeably reduced (12%).

COMPARISON OF PROLIFERATION INDEX WITH RESPECT TO ZONE AND PATHOLOGICAL CHANGES

The mean proliferation index from the normal peripheral zone was significantly less ($p < 0.01$) than all other areas: hyperplasia of the transition zone, peripheral zone atrophy, PIN, transition zone adenocarcinoma, and peripheral zone adenocarcinoma (table 3).

The areas of morphological atrophy (peripheral zone atrophy) showed a surprisingly high proliferation index, which was greater than normal peripheral zone and or hyperplastic transition zone ($p < 0.01$). There was no statistically significant difference between atrophy and PIN, and peripheral zone carcinoma.

The PIN proliferation index was not significantly different from either peripheral zone or transition zone carcinoma. The proliferation

Table 3 Statistical analysis

Zone proliferation indexes (mean (SE))				*n*	*Student's t test*	*Mann-Whitney test*
Normal peripheral zone (n = 16)	0.77 (0.08)	Hyperplasia of the transition zone	1.50 (0.12)	9	p < 0.01	p < 0.001
		Peripheral zone atrophy	4.80 (0.36)	13	p < 0.01	p < 0.001
		PIN	2.58 (0.29)	14	p < 0.01	p < 0.001
		Peripheral zone cancer	5.25 (0.34)	18	p < 0.01	p < 0.001
		Transition zone cancer	2.79 (0.28)	8	p < 0.01	p < 0.001
Peripheral zone atrophy (n = 13)	4.80 (0.36)	Hyperplasia of the transition zone	1.50 (0.12)	9	p < 0.01	p < 0.001
		PIN	2.58 (0.29)	14	p > 0.1	
		Peripheral zone cancer	5.25 (0.34)	18		p = 0.223
		Transition zone cancer	2.79 (0.28)	8	p > 0.01	
PIN (n = 14)	2.58 (0.29)	Peripheral zone cancer	5.25 (0.34)	18	p > 0.01	
		Transition zone cancer	2.79 (0.28)	8		p = 0.538
Peripheral zone cancer (n = 18)	5.25 (0.34)	Transition zone cancer	2.79 (0.28)	8		p < 0.001

Table 4 Immunocytochemical detection of proliferation index in the prostate

Reference	*Tissue studied*	*n*	*Technique*	*Findings*	*Labelling index (%)*		*Comments*
Cher *et al*[18]	Radical prostatectomy	30	BrdU (fresh)	Carcinoma	3.0		Only areas of carcinoma assessed. Reasonable correlation between the three techniques.
	TURP	6	PCNA (paraffin)		7.0		
	Needle biopsy	8	MIB-1 (paraffin)		3.4		
Hepburn *et al*[19]	TURP	20	Ki-67 (frozen)	Carcinoma	"H score"	0–0.51	Carcinoma only assessed, with staining score weighted according to intensity of stain. Good correlation between all three techniques.
			MIB-1 (paraffin)			0–1.35	
			PCNA (paraffin)			0–1.45	
Bonkhoff *et al*[20]	TURP(?)		Ki-67 (frozen)	Benign acini	Basal cells labelled		Benign tissue and PIN studied. Of the proliferating cells identified, basal cells contributed 70% in benign acini.
			MIB-1 (paraffin)	Hyperplasia	Basal cells labelled		
			PCNA (fresh)	PIN	Luminal cells labelled		
Grignon and Sakr[21]	Radical prostatectomy	39	MIB-1 (paraffin)	Transition zone cancer	1.6		Little difference between the mean Gleason grade for transition zone cancer (5.6) and peripheral zone cancer (6.5).
				Peripheral zone cancer	5.0		
Carroll *et al*[22]	Radical prostatectomy	32	BrdU (fresh)	Carcinoma	3.08 (sel)	1.62 (ran)	Labelling index assessed for selected areas of high staining (sel) and at random (ran).
	TURP	4	PCNA (paraffin)		6.02 (sel)	3.47 (ran)	
	Needle biopsy	12					
Henke[23]	Radical prostatectomy	11	MIB-1 (paraffin)	Atrophy	Up to 3 labelled cells/HPF		Quantitative labelling index not calculated. No difference between PCNA and MIB-1 scores.
			PCNA (paraffin)	Hyperplasia	Up to 3 labelled cells/HPF		
				Basal cell hyperplasia	Up to 15 labelled cells/HPF		
				Carcinoma	20-50 labelled cells/HPF		
Montironi *et al*[24]	"Prostatectomy" "Prostatic adenectomy"	80 in total	PCNA (paraffin)	Benign	3.16 (basal cells marking)		
				Atrophy	0.56 (non-basal cells)		
				PIN	9.5 (basal cells) 6.5 (non-basal cells)		
				Carcinoma	11.8 (mean value)		
Nemoto *et al*[25]	TURP Needle biopsy	21 in total	PCNA (paraffin)	Benign (7)	1.2		
				Atypical hyperplasia (2)	1.9, 4.1 (individual values)		
				Carcinoma (19)	7.6 (poorly differentiated)		
					4.6 (moderately differentiated)		
					2.5 (well differentiated)		
Sakr *et al*[26]	Radical prostatectomy	20	PCNA (paraffin, and fresh tissue for flow cytometry)	Carcinoma	8.55		Study also assessed AgNOR counts and flow cytometry.
Harper *et al*[27]	TURP	153	Ki-67 (frozen)	Carcinoma	Mean labelling index = 7.8% Labelling indexes ranged from 0 to 20%		High scores associated with poorly differentiated tumours.
Thompson *et al*[28]	TURP Benign	29	Ki-67 (frozen)	Benign	0.19		No significant correlation between tumour grade and labelling index.
	Carcinoma	34		Carcinoma	1.9		
Visakorpi[29]	Needle biopsy	93	PCNA (paraffin)	Carcinoma	9.7		
	TURP	48					
	Prostatectomy	23					
Raymond *et al*[30]	Needle biopsy & TURP Benign	10	Ki-67(frozen)	Benign	4		Significant correlation between tumour growth fraction and tumour grade.
	Carcinoma	21		Carcinoma	16.3		

HPF = high power field.

index of the transition zone carcinomas was approximately half that seen in peripheral zone carcinomas ($p < 0.01$). For peripheral zone cancers, Gleason sum score ranged between 5 and 8 (mean 6.5), and for transition zone cancers, it ranged between 4 and 8 (mean 5.4): this difference was not statistically significant ($p > 0.05$). No correlation between the proliferation

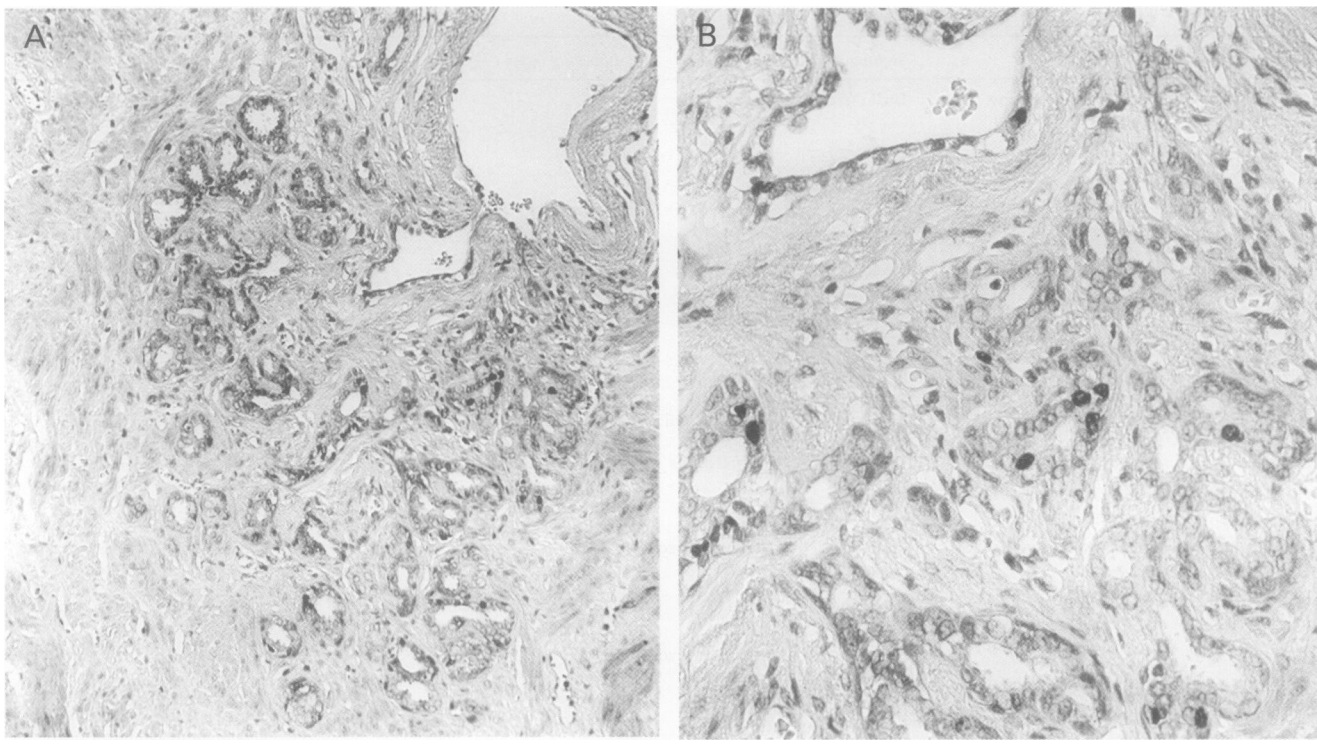

Figure 3 (A) Atrophic lobule associated with a dilated duct surrounded by fibrous tissue: frequent nuclei labelled with MIB-1. (B) Atrophy (higher power): double layer evident with luminal cells reduced in height.

index and tumour grade or tumour volume was observed, either when all tumours were considered collectively or when tumours of peripheral and transition zone origin were considered separately.

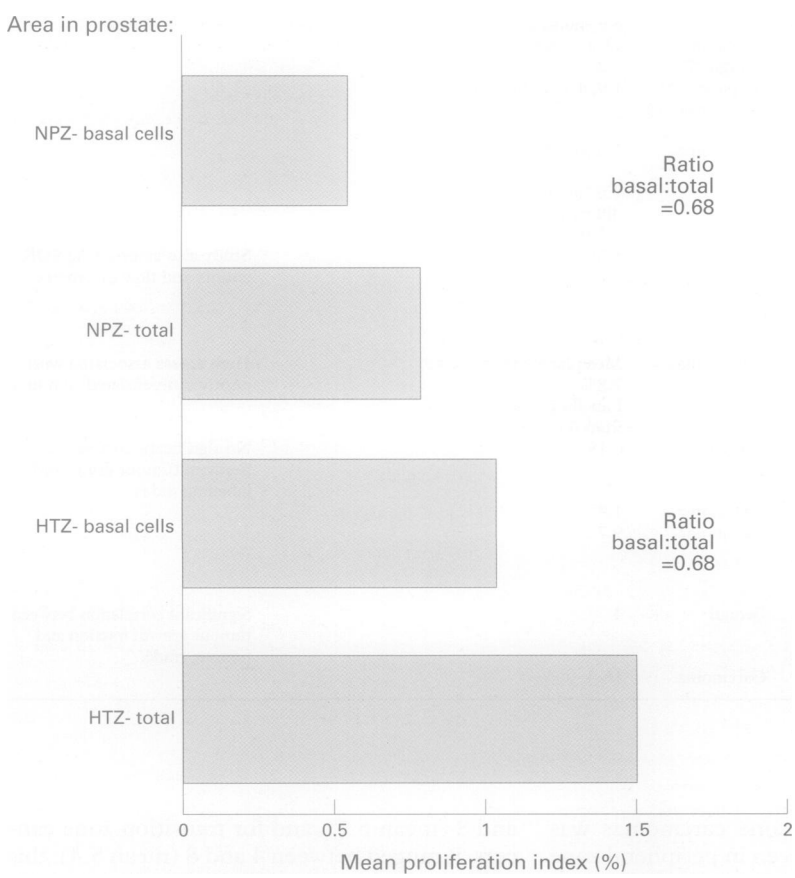

Figure 4 Contribution of basal cells to proliferation in non-neoplastic acini. NPZ = normal peripheral zone; HTZ = hyperplastic transition zone.

Discussion

Tumour growth is determined by the proportion of cells undergoing division, cell cycle time and the rate of cell death; the study of cell proliferation in tumours is of fundamental importance in understanding biological behaviour and may provide additional prognostic clinical information. Tumour kinetics have been investigated by mitotic counts, thymidine labelling, bromodeoxyuridine incorporation, AgNOR quantitation, and cytometric DNA analysis.[1-4] These techniques have been applied to the prostate,[9 15] but the use of antibodies active in paraffin wax processed tissue against cell proliferation markers now provides a simpler, more convenient means of estimating proliferation index. Furthermore, this has some prognostic value in other tumours.[16 17]

A summary of previous studies of cell proliferation (detected immunocytochemically) in the prostate is shown in table 4. Most of these studies have shown a significant correlation between proliferation markers and grade[24 25 27] and some found a significant association with clinical stage[22] or presence of metastases at presentation. The proliferation index of benign acini is consistently lower (0.19–4.0%) than that of malignant acini (1.6–16%). The values for transition zone carcinoma seem to be less than those quoted for peripheral zone tumour (1.6 as opposed to 5.6%). The values of the proliferation indexes obtained in this study all lie within the quoted ranges suggesting that within each study there is a reasonable degree of reproducibility, but that comparison of absolute values from different laboratories may not be valid. In the current study the observation that basal cells are the predominant proliferating cell in benign acini is in agreement with

other studies.[20 27] In contrast was the finding of an increased proliferation index in association with atrophy compared with other benign areas, which is at variance with other studies.[24]

In the TURP series, the tumour proliferation index correlated with both the grade and tumour stage. However, when logistic regression analysis was performed the Gleason sum score was the most reliable predictor of residual carcinoma on biopsy and tumour progression. The proliferation index did not provide any additional information. In contrast, in the radical prostatectomy specimens proliferation index was not associated significantly with grade or tumour volume. This might be explained by a possible underestimate of tumour volume in stage pT1a TURP cases, which is less likely in the radical cases. Furthermore, the tumour grade in the radical cases clustered around Gleason grade 2 and 3 and this factor, together with the small numbers of cases studied, may have obscured any underlying relation between grade and proliferation index.

In the radical prostatectomy specimens an important difference in proliferation index was related to the zone of origin of the carcinoma (transition zone or peripheral zone): the proliferation index in transition zone tumours was less than half that of peripheral zone tumours and was unrelated to grade. These findings are entirely consistent with Grignon and Sakr's observation[21] that transition zone cancer shows a lower proliferation index than peripheral zone cancer and may be correlated with the difference in morphology and behaviour seen in transition zone and peripheral zone cancers, with the better differentiated and less aggressive tumours arising in the transition zone.

The proliferation index in areas of high grade PIN was intermediate between that of normal tissue and invasive carcinoma. This again is in keeping with other published studies[24] and is consistent with the established view that PIN is a pre-malignant change from which carcinoma may arise.

It was of interest that apparent areas of focal lobular atrophy in the peripheral zone were associated with a significantly higher proliferation index than normal tissue and were within the range of peripheral zone carcinoma. In attributing significance to this finding we considered the results of two experimental studies using castrated rat prostate as a model for apoptosis. Initially, the increased expression of both PCNA and p53 in the ventral rat prostate was explained by the suggestion that apoptosis required cells to enter a defective cell cycle, allowing p53 to effect cell death.[31] According to this concept the high Ki-67 proliferation index in the areas of atrophy found in the radical prostatectomy cases would not reflect true cell proliferation. However, in a subsequent study, using a wider range of proliferation markers, including Ki-67, it was shown that the cells which underwent apoptosis had not entered the cell proliferation cycle.[32] Moreover, the atrophic rat prostate cells showed decreased expression of Ki-67. It would seem, therefore, that in our study the observed high rate of expression of MIB-1 in atrophic acini genuinely reflects a high rate of cell proliferation. This observation may be relevant to the long recognised spatial association between so-called atrophy and malignancy[33 34] and merits further investigation.

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