Urol Res (1997) 25:25–30

G. Chen · M.-S. Lin · R.-C. Li

Expression and prognostic value of proliferating cell nuclear antigen in transitional cell carcinoma of the urinary bladder

Received: 26 September 1995 / Accepted: 25 July 1996

Abstract Forty-eight patients with transitional cell carcinima (TCC) of the bladder were investigated. Routine paraffin-embedded sections were stained with proliferating cell nuclear antigen (PCNA) monoclonal antibody in order to determine the growth fraction of the bladder tumors and to correlate this with tumor grade, stage, development of recurrence and survival rate during follow-up. PCNA positive staining was detected in 95.8% (46/48) of the tumors. The mean labeling index (LI) of superficial tumors (Ta-1, $n = 28$) was 12.58 ± 12.33%, and 34.55 ± 21.89% in invasive tumors (T2-4, $n = 18$). A similar correlation was found in association with tumor grade. The patients were followed up for a mean of 4.9 years (range 1–14 years). The mean PCNA LI in nonrecurrent ($n = 21$) and simple recurrent ($n = 7$) superficial tumors was 11.29 ± 11.79% and 16.44 ± 14.05%, respectively, the difference not being statistically significant. To access survival, tumors with a PCNA LI above and below the median level (21%) were compared. Those patients ($n = 19$) with an index of > 21% (the mean of all the PCNA values) had a worse prognosis than those ($n = 27$) with an index of < 21%, a difference which is statistically significant. These results suggest that PCNA LI in bladder cancer may prove to be an objective and quantitative assay of biological aggressiveness and provide significant prognostic information, although it does not help the selection of patients at risk of simple recurrence in superficial tumors.

G. Chen (✉) · R.-C. Li
Department of Urology, Jinshan Hospital,
Shanghai Medical University, Shanghai, 200540,
People's Republic of China

M.-S. Lin
Department of Pathology, Jinshan Hospital,
Shanghai Medical University, Shanghai, 200540,
People's Republic of China

Introduction

Predicting future tumor behavior has always been a major task when treating bladder cancer, but it has remained poorly understood to date. Histological grade and stage are the most frequently used predictors of prognosis. However, the criteria for placing a tumor in a particular grade are somewhat subjective and the behavior of the tumor is not always accurately predicted by these means.

Cell kinetics information has become increasingly important as a potential prognostic indicator in a variety of tumors [2, 8, 11, 13, 15, 17, 19, 20–24]. The rate of cell proliferation is believed to be a major influence of tumor behavior and it has been shown that proliferative activity correlates with tumor recurrence and invasive growth, metastatic potential and, in some instances, overall prognosis. Several methods have been used to evaluate proliferative activity, including flow cytometry [2, 20], morphometric analysis [3, 18], cytochemical silver-staining (argyrophilic nucleolar organizer scores) [7, 12, 16], and immunohistochemical staining of proliferating cell nuclear antigen (PCNA) or ki-67 antigen [8, 11, 19, 22–24] and incorporated bromo- or iododeoxyuridine (in vitro or in vivo) [13, 15, 17, 21]. However, most of these methods are complex and/or costly, which limits their widespread use in clinical practice.

PCNA, also known as cyclin, is an auxiliary protein of DNA polymerase delta and plays a critical role in the initiation of cell proliferation [4, 14]. It was first discovered in patients with systemic lupus erythematosis, who produce an autoantibody to it [1]. The expression of PCNA is closely linked to the cell cycle. Evaluated levels of PCNA appear in the nucleus during the late G1 phase immediately before the onset of DNA synthesis, become maximal during the S phase, and decline again during G2 and M phases. Its expression is correlated with the S phase of the cell cycle and the cell proliferative status. It is recognized by PC10, a monoclonal antibody to PCNA, in routinely processed paraffin wax embedded

tissues, and it has been suggested that PCNA levels may be of value as an independent prognostic variable [8, 11, 19, 24].

We compared the cell kinetic data, defined by PCNA LI, with morphological grade and stage, and evaluated the role of PCNA LI as a prognostic indicator in TCC of the bladder in a retrospective study of 48 patients and report our results.

Material and methods

Between 1979 and 1993, 48 patients with TCC of the bladder underwent curative resection at our institution, including TURBT(15), partial cystectomy(19) and radical cystectomy(14). There were 44 men and 4 women (ratio 11:1, mean age 58.5 years, range 30–85 years). According to operative and pathological reports and metastasis classification of the International Union Against Cancer, tumors were classified as Ta tumor when limited to the mucosal layer, T1 tumor when invading the submucosal layer, T2 tumor when invading the superficial muscle, T3 tumor when invading deep muscle and T4 tumor when invading the membranes and surrounding tissue. Specimens were graded according to the World Health Organization system. Follow-up ranged from 1 to 14 years (mean 4.9 years) and 5-year follow-up was available with 22 of the 48 patients.

The excised specimens were routinely fixed with formalin and embedded in paraffin wax blocks. Sections of 5 μm were cut and mounted on glass slides. PCNA staining was performed with the standard ABC immunoperoxidase technique, and five biopsies taken from the patients with benign prostate hypertrophy or urinary stone disease served as normal controls. Monoclonal antibody PC10 to human PCNA was obtained from the Dako Corporation (code No:M879). All PCNA staining was performed in one batch in order to avoid interbatch variation.

Each section was counted manually at high power (×400) after identifying at low power (×100) the representative areas with the highest concentration of stained cells according to the recommendation of Cohen et al. [5]. About 1000 cells/slide were counted in each of five to ten microscopic fields from well-labeled areas to determine the average PCNA LI. Malignant cells were counted consecutively moving from right to left and then down as in reading a page. In low-grade superficial tumors, positive cells tended to be located in the basal cell layer of the epithelium, whereas in grade 2–3 tumors positive cells were present throughout the layers. PCNA LI was expressed as number of labeled cells (positive for PCNA) as a percentage of the total number of cells counted in each specimen. All identifiable staining was regarded as positive [6]. All cases were counted by one pathologist in order to avoid interobserver error, who had no knowledge of the clinicopathological data.

The results are expressed as mean plus or minus standard deviation. For statistical analysis, the *t*-test was used to compare groups of patients. A p value < 0.05 was considered significant. The effect of PCNA levels on survival was assessed by comparing the groups using Kaplan-Meier survival curves with statistical significance determined by the log-rank test.

Results

PCNA-positive staining was detected in 95.8% (46/48) of the tumors, but in none of the normal controls. Two cases with PCNA-negative staining were excluded from this study. An example of PCNA staining is given in Figs.1 and 2. The mean PCNA LI in tumors of different grade and stage are summarized in Table 1. The differences of PCNA LI between tumors of different grade and stage were statistically significant.

To compare PCNA LI and recurrence, 7 out of 28 superficial tumors had simple recurrence (without progression in grade and stage), while 21 did not recur. The mean PCNA LIs (at diagnosis) in recurrent and

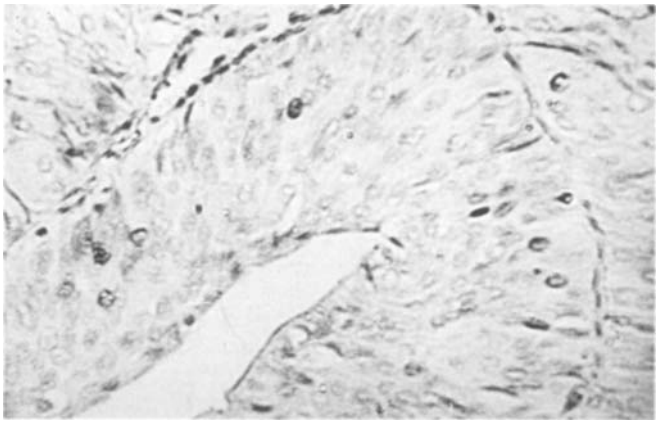

Fig. 1 G1 TCC immunoperoxidase stain for PCNA shows a few labeled turnor nuclei in the basal cell layer, × 400

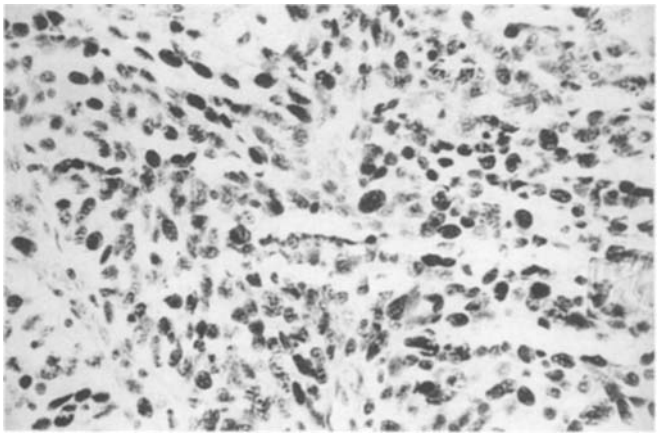

Fig. 2 G3 TCC immunoperoxidase stain for PCNA shows many labeled turmor nuclei throughout the layers, × 400

Table 1 Correlation between PCNA labeling index and clinical and pathological variables

	Patients (n)	PCNA labeling index (mean ± SD%)	P
All tumors	46	21.18 ± 19.74	
Grade			
1	18	8.28 ± 8.68*	
2	20	23.82 ± 16.02**	
3	8	44.68 ± 24.65	
Stage			
Ta-1	28	12.58 ± 12.33	
T2-4	18	34.55 ± 21.89	$P < 0.001$

* Significantly ($P < 0.001$) lower than G2, G3
** Significantly ($P < 0.05$) lower than G3

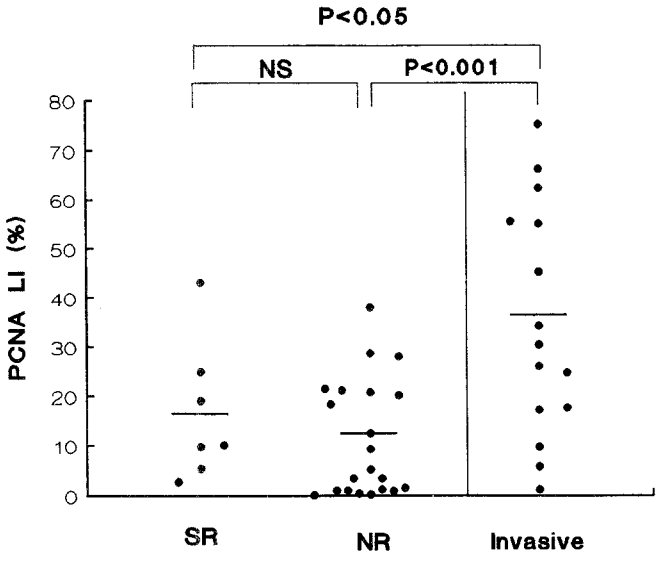

Fig. 3 PCNA LI in relation to recurrence. SR simple recurrence, NR non-recurrence. *Bars* indicate mean PCNA LI. *NS* not statistically significant

nonrecurrent tumors were 16.44 ± 14.05% and 11.29 ± 11.79%, respectively, the difference not being statistically significant. Of 18 invasive tumors, 15 cases recurred when transurethral resection as the first choice of treatment was attempted, and most recurrences were with progression. Therefore 14 cases eventually needed radical cystectomy. The mean PCNA LI (at diagnosis) in recurrent invasive tumors was 35.88 ± 22.52% and this was statistically significant when compared with non-invasive tumors (Fig 3).

To compare PCNA index and survival, patients were divided into two groups, depending on the level of PCNA staining [9]. Nine of the 19 patients with a PCNA count > 21% (the mean of all the PCNA values) died of bladder cancer, and 1 died of another cause. In the group with a PCNA count < 21% (27 patients), 3 died of bladder cancer, and 5 died of other causes. The Kaplan-Meier survival curves of these two groups are shown in Fig 4. Patients with a PCNA count > 21% (the mean of all the PCNA values) had a worse prognosis than those with a PCNA count < 21% ($P < 0.05$).

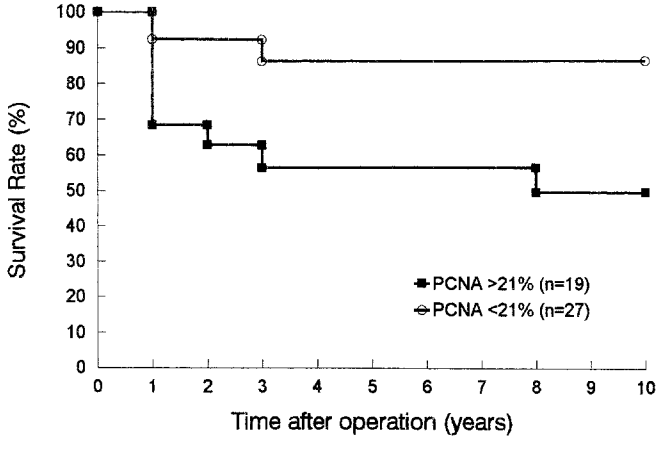

Fig. 4 Kaplan-Meier survival curve comparing survival of 27 (⊖) and 19 (-■-) patients with PCNA LIs of less than and more than 21%, respectively. Cutoff level of 21% represents the mean of all the PCNA values (log-rank test, $P < 0.05$)

Discussion

One of the problems in the management of patients with bladder cancer – perhaps the main problem – is the difficulty in predicting the behavior of a bladder tumor in an individual case. Even after complete transurethral resection (TUR), 50–80% of tumors recur and up to 30% progress in stage or grade [10]. If there were some means of identifying those tumors most likely to go on to being invasive, and conversely those least likely to do so, one could institute the most appropriate form of therapy at a much earlier stage.

Many prognostic factors have been investigated, for example, age, tumor size, multifocality, presence of concomitant carcinoma in situ, histological grade and stage are among the many well-established means used to predict outcome. Nevertheless, ideally one requires some more precise way of identifying those tumors which are likely to pursue an aggressive course with deep invasion and perhaps metastasis. It is against this background that many other methods have been investigated recently, e.g., determination of blood group isoantigens, chromosomal analysis, tumor suppressor gene, tumor-associated antigens and cell proliferative rates. Molecular classification of tumors is a particularly promising approach for predicting biological activity.

Several reports have suggested that PCNA may have clinical applications in the histological grading of tumor and as an independent prognostic variable in certain malignances [8, 11, 19, 24]. These reports prompted us to examine PCNA in TCC of the bladder to determine its value in this tumor. The PCNA monoclonal antibody, PC10, is reliably incorporated into cellular nuclei at the time of DNA synthesis prior to mitosis, as would be expected from a reliable S phase marker which allows quantitative study. This was an important breakthrough for the studies of cellular kinetics. Failed PCNA staining in two tumor specimens in our study might be due to the duration of fixation in formalin longer than 72 h, which destroyed the epitope [8].

There was some variation in the intensity of the nuclear staining around strongly positive cells. Cells G1, G2 and mitosis are reported to exhibit weak staining with the PCNA antibody, while a strong staining pattern is observed as the cells progress through the S phase. In our study, all identifiable staining was regarded as positive according to Cronin et al. [6]. This might be the reason that the PCNA LIs in our study were higher than those of Hattori et al. [9].

When heterogeneity of cellular anaplasia was present in a tumor, the worst grade present was assigned to

that specimen. Similarly, when heterogeneity of PCNA staining was present between different fields, the representative areas with the highest concentration of stained cells were counted in order to minimize interstaining variations [5]. Sampling is important when the PCNA LI is used as a prognostic study of bladder cancer [9].

In our study, PCNA LI has been shown to correlate well with histological variables, such as grade or stage. This finding probably suggests that proliferative activity defined by PCNA LI may be important in control of tumor behavior.

In survival analysis, we found that PCNA LI (at diagnosis) correlated significantly with survival. Those patients with an index of > 21% (the mean of all the PCNA values) had a worse outcome than those with an index of < 21%. It may be of clinical significance in that it may allow urologists to identify patients with identical tumor stage and grade who have a worse prognosis and enable them to institute radical or adjuvant therapies accordingly. This elimination of delay may ultimately improve the efficacy of current therapies.

In superficial tumors, none of the 28 patients developed invasive growth, although there were simple recurrences in 7 of those patients. The differences in PCNA LI in superficial tumors between nonrecurrence and simple recurrence were not statistically significant. Surprisingly, there was no recurrence with progression in superficial tumors in our series. We suppose that some of these were originally superficial ones, but they had progressed to invasive growth early in the clinical history when first diagnosed. Our findings were similar to those of Hattori et al. [9]. Therefore, PCNA expression probably represents only the biological aggressiveness of the primary tumors. Our data may provide further support for this view. For the identification of reliable markers of simple recurrence in superficial tumors, other approaches from several different aspects will be needed. For example, the in vivo labeling of bromodeoxyuridine and ki-67 staining in TCC of the bladder have been found to be useful in predicting recurrence [17, 21], it would appear reasonable from this assumption that initially superficial tumors with low PCNA expression are unlikely to proceed to invasion, whereas in cases with high PCNA expression they probably will do so.

In conclusion, our results show that PCNA expression mirrors the biological aggressiveness in TCC of the bladder and it has a positive association with tumor grade, stage and survival, although it does not help to select patients at risk of simple recurrence for superficial tumors. We believe it can give additional help when used in association with standard prognostic criteria for the management of patients with bladder cancer. However, this would require confirmation in a larger number of patients and longer follow-up in order to define the PCNA index range that will be indicative of future tumor behavior.

References

1. Asero R, Origgi L, Crespi S, Bertetti E, D'Agostino P, Riboldi P (1987) PC autoantibody to proliferating cell nuclear antigen(PCNA) in SLE: a clinical and serological study. Clin Exp Rheumatol 5:241
2. Barlogie B, Raber MN, Schumann J, Johnson TS, Drewinko B, Swartzendruber DE, Gohde W, Andereeff M, Freireich EJ (1983) Flow cytometry in clinical cancer research. Cancer Res 43:3982
3. Borland RN, Partin AW, Epstein JI, Brendler CB (1993) The use of nuclear morphometry in predicting recurrence of transitional cell carcinomas. J Urol 149:272
4. Bravo R, Frank R, Blundell PA, MacDonald-Bravo H (1987) Cyclin/PCNA is the auxiliary protein of DNA polymerase delta. Nature 326:515
5. Cohen MB, Waldman FM, Carroll PR, Kerschmann R, Chew K, Mayall BH (1993) Comparison of five histopathologic methods to assess cellular proliferation in transitional cell carcinoma of the urinary bladder. Hum Pathol 24:772
6. Cronin KJ, Williams NN, Kerin MJ, Creagh TA, Dervan PA, Smith JM, Fitzpatrick JM (1994) Proliferating cell nuclear antigen: a new prognostic indicator in renal cell carcinoma. J Urol 152: 834
7. Dervan PA, G:lmartin LG, Loftus BM, Carney DN (1989) Breast carcinoma kinetics: Argyrophilic nucleolar organizer region counts correlate with ki-67 scores. Am J Clin Pathol 92:401
8. Hall PA, Levison DA, Woods AC, Yu CC-W, Kellak DB, Watkins JA, Barnes DM, Gillett CE, Camplejohn R, Dover R, Waseem NH, Lane DP (1990) Proliferating cell nuclear antigen (PCNA) immunolocalization in paraffin sections: an index of cell proliferation with evidence of deregulated expression in some neoplasms. Pathology 162:285
9. Hattori K, Uchida K, Akaza H, Koiso K, Nemoto R, Harada M (1995) Proliferating cell nuclear antigen cyclin in human transitional cell carcinoma. Br J Urol 75: 162
10. Henry NM, Nocks BN, Daly JJ, Prout GR, Newall JB, Griffin P, Perrone T, Szyfelbein W (1982) Ta and T1 bladder cancer: location, recurrence and progression. Br J Urol 54: 152
11. Lipponen PK, Eskelinen MJ (1992) Cell proliferation of transitional cell bladder tumors determined by PCNA/cyclin immunostaining and its prognostic value. Br J Cancer 66:171
12. Lipponen PK, Eskelinen MJ, Nordling S (1991) Nucleolar organizer regions as prognostic factor in transitional cell bladder cancer. Br J Cancer 64:1139
13. Ljunberg B, Larsson P, Roos G, Stenling R,Wilson G (1994) Cell kinetics of renal cell carcinoma studied in vivo iododeoxyuridine incorporation and flow cytometry. J Urol 151:1509
14. Mathews MB, Bernstein RM, Franza BR, Garrels JL (1984) Identity of the proliferating cell nuclear antigen and cyclin. Nature 303:374
15. Nemoto R, Hattori K, Uchida K, Shimazui T, Nishijima Y, Koiso K, Harada M (1990) S phase fraction of human prostate adenocarcinoma studied with in vivo bromodeoxyuridine labelling. Cancer 66:509
16. Pich A, Valente G, Azzomi L, Stramignoni A, Margaria E, Tasso M (1991) Argyrophilic nucleolar organizer region counts and ki-67 scores in human renal cell carcinoma. Pathol Res Pract 187:482
17. Popert RJM, Joyce AD, Thomas DJ, Walmsley BH, Coptcoat MJ (1993) Bromodeoxyuridine labelling of transitional cell carcinoma of the bladder – an index of recurrence? Br J Urol 71:279
18. Portillo JA, Val-Bernal JF, Garijo MF, Buelta L, Gutierrez JL (1992) Prognostic correlation of morphometric value with survival in invasive transitional cell carcinoma of bladder. Br J Urol 70:628
19. Robbins BA, De La Vega D, Ogata K, Tan EM, Nakamura RM (1987) Immunohistochemical detection of proliferating cell

nuclear antigen in solid human malignancies. Arch Pathol Lab Med 11:841
20. Sowa M, Yoshino H, Kato Y, Nishimura M, Kamino K, Umeyama K (1988) An analysis of the DNA ploidy patterns of gastric cancer. Cancer 62:1325
21. Tsujihashi H, Nakanishi A, Matsuda H, Uejima S, Kurita T (1991) Cell proliferation of human bladder tumors determined by BrdUrd and ki-67 immunostaining. J Urol 145:846
22. Van Dierendonck JH, Keijizer R, Van de Velde, CJH, Cornelisse CJ (1989) Nuclear distribution of the ki-67 antigen during the cell cycle: comparison with growth fraction in human breast cancer cells. Cancer Res 49:2999
23. Yonemura Y, Ooyama S, Sugiyama k (1990) Growth fraction in gastric carcinomas determined with monoclonal antibody ki-67. Cancer 65:1130
24. Yu CC-W, Hall PA, Fletcher CDM, Camplejohn R, Waseem NH, Lane DP, Levison DA (1990) Immunohistochemical staining with a monoclonal antibody to PCNA may be a good indicator of prognosis in haemangiopericytomas. J Pathol 161:342a