Eur J Cancer, Vol. 28A, No. 6/7, p. 1296, 1992.
Printed in Great Britain
0964-1947/92 $5.00 + 0.00

Comparison of Growth Fraction with Tumour Stage and Grade in Renal Cell Carcinoma

W. de Riese, E. Allhoff, S. Liedke, M. Werner, H. Kirchner and J. Atzpodien

110 DIFFERENT renal cell carcinomas (RCC) were investigated with immunohistostaining with the monoclonal antibody (Mab) (Ki-67) which allows direct determination of growth fractions of normal as well as malignant tissue *in situ* [1–4].

The tumours came from patients with no metastatic disease at nephrectomy. Different random biopsy specimens from normal and malignant renal tissue were taken. Each sample was divided for routine staining and for storage in liquid nitrogen. Cryostat sections were prepared [5, 6]. The determination of Ki-67-positive cells was as described [7]. To exclude sampling errors, several samples of solid tumour must be obtained, depending on the individual tumour size [6]. Several biopsies are necessary to prevent errors arising from intratumoral variability [4]. The highest proliferation rate for all specimens in a given case is representative of the entire tumour [4, 6].

The growth fractions (percentage of Ki-67 positive cells) of all malignant renal tumours analysed in our study ranged between 1 and 19%, while normal renal tissue had growth fractions up to 2% only. TNM classification was done according to the current UICC criteria [8]. Tumour infiltrating lymphocytes often observed in the peripheral zones of RCC should be excluded in the immunohistochemical Ki-67 evaluation. These lymphocytes exhibit cell proliferation within the tumour and can therefore simulate higher growth fractions of the tumour. The proliferation of tumour infiltrating lymphocytes might be caused by lymphokines directly. To exclude this on frozen sections, reference slides of each specimen must be taken for conventional histology; histological differentiation between lymphocytes and tumour cells is almost impossible on frozen sections alone [9]. Ki-67 immunohistostaining cannot be done on paraffin sections because paraffin embedding destroys the Ki-67 nuclear antigen [2, 10].

No correlation between individual growth fractions and tumour stage (pT; Fig. 1) was found. In low stage tumours (pT 1–2), a 5-fold variation in growth fraction was measured. Several small tumours (< 3 cm in diameter) had a growth fraction over 9%, whereas some large tumours showed a low growth fraction.

In contrast, a strong correlation between growth fraction and low grade (G1) as well as high grade (G3) RCC tumours was observed (Fig. 1). According to these data, the individual tumour stage cannot be deduced from an individual growth fraction, as stage is a function of individual cell proliferation as well as of individual age of a given tumour, which is not measurable at time of diagnosis. These results correspond with previous data in breast cancer: a positive correlation between histological grading and growth rate of tumour tissue was observed [3, 10].

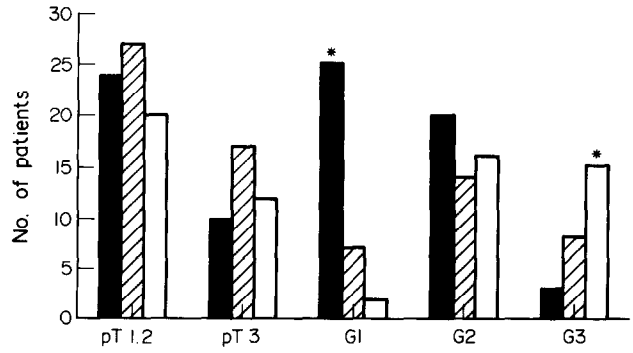

Fig. 1. Distribution of growth fractions in 110 RCCs of different stages (pT) and grades (G) without evidence of metastatic disease at time of nephrectomy. Growth factor: ■ < 4%, □ 4–9%, □ > 9%. *$P < 0.05$ χ^2.

Immunohistochemical Ki-67 determination of tumour specific growth fractions in RCC is practicable, reliable and reproducible. In our ongoing study the growth fractions are being correlated with follow-up. According to the preliminary results, measurement of the individual growth fractions appears to be an additional diagnostic index for the detection of RCC patients at high risk, especially at early stages showing identical histological features.

1. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer* 1983, **31**, 13–20.
2. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol* 1984, **133**, 1710–1715.
3. Stenfert Kroese MC, Rutgers DH, Wils IS, Van Unnik JAM, Roholl PJM. The relevance of DNA index and proliferation rate in the grading of benign and malignant soft tissue tumors. *Cancer* 1990, **65**, 1782–1788.
4. Simony J, Pujol JL, Radal M, Ursule E, Michel FB, Pujol H. *In situ* evaluation of growth fractions determined by monoclonal antibody Ki-67 and ploidy in surgically resected non-small cell lung cancers. *Cancer Res* 1990, **50**, 4382–4387.
5. Cordell JL, Falini B, Erber NW, *et al.* Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1985, **32**, 219–229.
6. de Riese W, Allhoff E, Stief CG, *et al. In vitro* sensitivity testing of human renal cell carcinoma with cytostatic agents and interferon alpha-2a. *Urol Res* 1991, **19**, 87–90.
7. Okamura K, Miyake K, Koshikawa T, Asai J. Growth fractions of transitional cell carcinomas of the bladder defined by the monoclonal antibody Ki-67. *J Urol* 1990, **144**, 875–878.
8. Hermanek P, Sobin LH. Classification of urological tumors. In: Hermanek P, Sobin LH, eds. *TNM Classification of Malignant Tumors*. Berlin, Springer, 1987, 137–139.
9. de Riese W, Allhoff E, Werner M, *et al.* Proliferative behaviour and cytogenetic changes in human renal cell carcinoma. *W J Urol* 1991, **9**, 79–85.
10. Lelle RJ, Heidenreich W, Stauch G, Gerdes J. The correlation of growth fractions with histologic grading and lymph node status in human mammary carcinoma. *Cancer* 1987, **59**, 83–88.

Acknowledgements—We thank Dr Terry Reister, Department of Biostatistics, Indiana University for statistical advice and Dr Sam Little, Department of Urology, Indiana University for reading the manuscript.

Correspondence to W. de Riese.
W. de Riese, E. Allhoff and S. Liedke are at the Department of Urology; M. Werner is at the Laboratory of Cytogenetics and Immunohistochemistry, Institute of Pathology; and H. Kirchner and J. Atzpodien are at the Department of Hematology and Oncology, Hannover Medical School, Konstanty-Gutschow-Str. 8, 3000 Hannover 61, Germany.
Revised 22 Dec. 1991; accepted 15 Jan. 1992.