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EXCRETION OF PSEUDOURIDINE AS AN INDEPENDENT PROGNOSTIC FACTOR IN RENAL CELL CARCINOMA

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Abstract

Pseudouridine is the most prevalent modified nucleoside excreted in urine, mainly as a degradation product of t-RNA. The level of pseudouridine excretion was analyzed in 71 patients with renal cell carcinoma prior to treatment. An increased excretion was demonstrated in 27 of 48 patients (56%) in stage II-IV, compared to 2 of 23 patients (9%) in stage I. Survival time was significantly reduced in patients with increased excretion. The level of pseudouridine correlated to tumor grade and tumor size. Using Cox's proportional hazard model, only clinical stage and level of pseudouridine excretion were independent predictors of prognosis.

Key words: Renal cell carcinoma, pseudouridine, modified nucleosides, prognosis.

Ribonucleic acid (RNA) consists of a chain of nucleotides, some of which are modified. This modification takes place after the synthesis of the polynucleotide chain and is a specific process. The modification is most frequent in transfer-RNA (t-RNA), and the most prevalent modified nucleoside is pseudouridine. There is no salvage pathway for the modified nucleosides and they are excreted in the urine upon degradation of the RNA.

Increased excretion of modified nucleosides has been observed in patients with various malignant diseases (1–7), and also in some benign diseases (8, 9). Children normally have a higher excretion of modified nucleosides than adults (10, 11). It has been proposed that the level of modified nucleosides in urine is a measure of the rate of turnover of t-RNA in the organism (12).

No clinically useful tumor marker has yet been identified in patients with renal cell carcinoma (13). The strongest predictor of prognosis is the clinical stage (14), but DNA content, tumor grade and cell type are also important prognostic parameters (14–16).

The aim of the present study was to assess the putative prognostic value of psuedouridine excretion in urine from patients with renal cell carcinoma.

Material and Methods

Seventy-one patients with renal cell carcinoma were treated at the Departments of Urology and Oncology, University Hospital, Umeå, Sweden. There were 37 males and 34 females. Their median age was 64 years (range 28–86). Upon admission a physical examination was performed, and hematologic status assessed. The patients were examined with chest radiography, computerized tomography (CT), ultrasonography (US), and most of them with angiography and bone scintigraphy. The size of the tumors was measured either by CT or by angiography using a 17% size reduction due to the magnification.

The patients were staged according to Robson et al. (14). Twenty-three patients had tumors confined to the kidney (stage 1), 16 had tumors invading the perirenal fat, the renal vein and/or the regional lymph nodes (stage II-III), and 32 had distant metastases (stage IV).

Fifty-six patients were operated upon with perifascial nephrectomy, 7 were treated with arterial occlusion and 8 had other palliative treatments. Morphologic examination was performed in 61 patients according to Skinner et al. (17) using a four-graded scale.

Pseudouridine. Urine samples were collected at admission and stored at −70°C until analysis. The concentration of pseudouridine was determined according to Gehrke

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et al. (18) and Kuo et al. (19), using high performance liquid chromatography. Pseudouridine was correlated to the creatinine concentration in the sample, determined by the Technicon procedure. A reference group was chosen, consisting of 79 healthy adults, evenly distributed as to sex and aged from 17–79 years (median 46). Based on this group the reference levels were set at the mean value +2 SD, giving 30 and 35 nmol/μmol of creatinine for males and females respectively.

The patients were followed 30 to 80 months (median 54 months) from admission. During this period 43 patients died, 39 due to renal cell carcinoma and 4 of intercurrent diseases. Twenty-eight patients were alive at the end of the follow-up period. Survival time was calculated from the time of admission.

The Student's t-test and Wilcoxon's test were used for statistical analysis and the survival time calculations were done according to Kaplan & Meier using the log rank test (20). Multivariate analysis was performed according to Cox's proportional hazard model (21).

Results

Reference material. Females had slightly higher excretion of pseudouridine than males. The median values were 23.0 and 19.3 nmol/μmol of creatinine respectively (Table 1). When these individuals were subdivided into different age groups, it revealed a slightly higher excretion in persons above 70 years of age compared to those below 70. This difference was not statistically significant.

Clinical material. There was a statistically significant elevation of pseudouridine excretion between stage I and II-III (Table 2), but no further increase was observed in stage IV. This was found for both males and females. A correlation was also demonstrated between pseudouridine level and tumor grade as shown in Table 3.

When tumor size in patients without distant metastases (stage I-III) was related to pseudouridine excretion (Fig. 1), it appeared that males had larger tumors than females. There was a correlation between pseudouridine excretion and tumor size ($r = 0.3898$, $p = 0.02$).

Survival. Twenty-eight patients were alive after a median observation time of 54 months. In stage IV the median survival time of 16 patients with pseudouridine excretion levels below the reference limit was 19 months compared to 6 months for 16 patients with elevated excretion (Table 4). This difference is statistically significant ($p = 0.005$). Patients in stage I and II-III are presented together in Fig.

Table 1

Pseudouridine excretion in 79 healthy adults

	Total No.	Pseudouridine				Elevated	
		mean	median	SD	range	No.	(%)
Males	41	20.0	19.3	5.06	11.6–34.4	2	5
Females	38	22.7	23.0	6.13	7.8–34.7	–	–

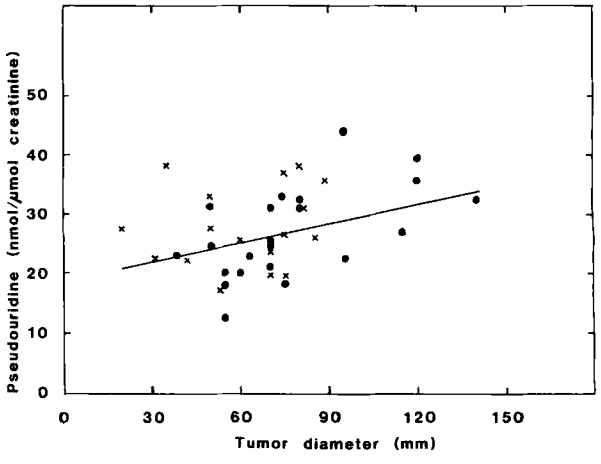

Fig. 1. Tumor size in relation to pseudouridine excretion in 39 patients in stages I-III. ●: males (n = 22); ×: females (n = 17). r = 0.390.

Table 2

Pseudouridine excretion in relation to clinical stage in 71 patients with renal cell carcinoma

	Total No.	Pseudouridine				Elevated		
		mean	median	SD	range	No.	(%)	(%)
Stage I								
Males	10	21.5	22.6	4.18	12.3–26.8	–	–	9
Females	13	25.9	25.4	5.91	17.2–37.8	2	15	
Stage II-III								
Males	12	31.3	31.7	6.88	19.9–44.0	9	75	69
Females	4	33.6	35.2	4.73	26.0–37.9	2	50	
Stage IV								
Males	15	30.9	28.2	10.27	18.8–55.0	7	47	50
Females	17	35.0	35.4	11.74	20.3–63.5	9	53	

Table 3

Pseudouridine excretion in relation to histopathologic grade in 61 patients with renal cell carcinoma

	Total No.	Pseudouridine				Elevated		
		mean	median	SD	range	No.	(%)	(%)
Grade 1–2								
Males	7	19.2	19.4	3.42	12.3–23.2	–	–	8
Females	6	25.6	25.7	6.67	17.2–37.8	1	17	
Grade 3								
Males	17	30.2	30.9	8.52	18.2–55.0	10	59	44
Females	17	29.0	26.3	6.66	19.7–43.5	5	29	
Grade 4								
Males	9	28.8	26.8	7.15	19.9–24.6	3	33	57
Females	5	42.1	37.2	10.72	35.8–63.5	5	100	

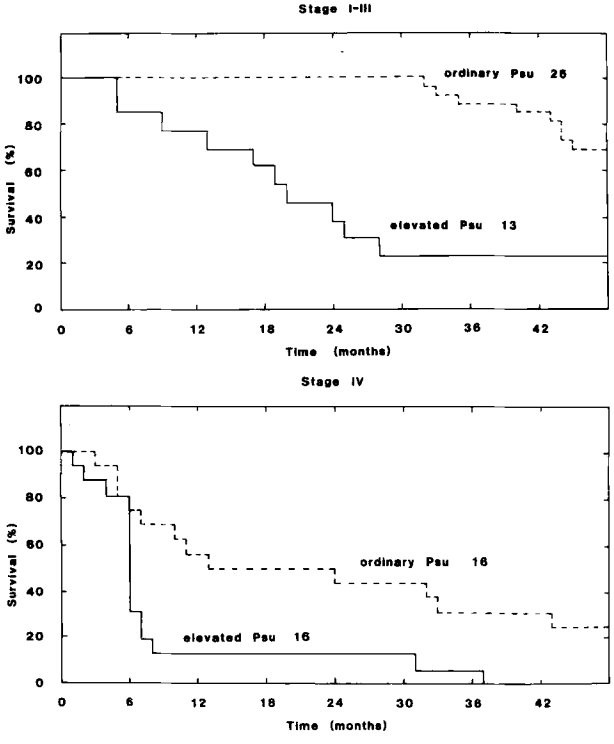

Fig. 2. Survival of 39 patients in stages I-III (upper panel), and of 32 patients in stage IV (lower panel). Patients with ordinary (– – –), and elevated excretion (——) of pseudouridine before treatment.

2, since survival times were similar in these stages, as shown in Table 4. In patients in stage I-III too, an increased excretion of pseudouridine correlates with a poorer prognosis of survival ($p = 0.001$). There was no significant difference in age or sex ratio between patients with ordinary and elevated excretion of pseudouridine in the compared groups.

Multivariate prognostic analysis. The relation between survival time, age, sex, tumor size, grade, clinical stage and pseudouridine excretion was analyzed using Cox's proportional hazard regression model. Only two parameters were independently predictive of prognosis, namely clinical stage and the level of pseouridine excretion (Table 5).

Table 4

Survival of 71 patients with renal cell carcinoma in different clinical stages in relation to initial pseudouridine excretion

	Total No.	Survival (months)			Alive	
		mean	median	range	No.	(%)
Stage I						
Ordinary PSU	21	55.6	54+	32–82+	19	90
Elevated PSU	2	24.5	25	24–25	–	–
Stage II-III						
Ordinary PSU	5	52.5	54+	40–67+	5	100
Elevated PSU	11	29.7	19	5–80+	2	18
Stage IV						
Ordinary PSU	16	26.0	19	3–70+	2	13*
Elevated PSU	16	9.1	6	1–37	–	–

PSU = pseudouridine *($p = 0.005$).

Discussion

Earlier reports concerning the excretion of pseudouridine and other modified nucleosides have shown that the highest frequency of elevated excretion was found in patients with tumors with a high proliferative growth fraction, e.g. malignant lymphomas, leukemias and small cell carcinoma of the lung (1, 3–5). Reports have also shown that the excretion was reduced following successful chemotherapy of these diseases (3–5). Furthermore, the excretion is high in healthy children (10, 11). An elevated excretion of modified nucleosides could be considered as a marker of cell proliferation rather than a general marker of malignancy.

To our knowledge there are only a few reports dealing with the excretion of modified nucleosides in renal cell

Table 5

Multivariate analysis of prognostic factors according to Cox

Prognostic factor	Risk estimate	SD	p	95% conf. interv.
Stage I-III versus IV	2.17	0.44	0.00	1.30–3.04
Pseudouridine Ordinary versus elevated	1.68	0.44	0.00	0.82–2.54
Size <60 mm versus >60 mm	0.80	0.49	0.10	−0.16–1.75
Age <65 versus >65 years	0.33	0.42	0.44	−0.50–1.15
Sex male versus female	0.17	0.37	0.65	−0.55–0.89
Grade 1–2 versus 3–4	−0.16	0.65	0.81	−1.43–1.12

carcinoma (22, 23). Koshida et al. (23) analyzed pseudouridine and three other modified nucleosides in 8 patients with renal cell carcinoma. The excretion of most of the nucleosides was reduced after nephrectomy. Two of the patients with lung metastases at the time of operation had a continuously increased excretion of 1-methylinosine. Consecutive sampling from one patient with disease progression showed increasing excretion of pseudouridine and 1-methylinosine over a period of two months.

Our results demonstrate a moderate correlation between tumor size and the level of pseudouridine excretion. It should, however, be pointed out that a 64-fold increase in tumor volume only corresponds to a doubling of the pseudouridine excretion.

Numerous reports have been made concerning prognostic factors in renal cell carcinoma (14–17, 24–25). The strongest predictor of prognosis is the clinical stage (14, 16, 24). This observation is supported by our results. The histologic grade and DNA content are also of significance according to univariate prognostic analysis (14–17). However, using Cox's proportional hazard model, Lieber et al. (25) found that only stage and weight loss before operation were independent prognostic factors in young adults. Using the same statistical analysis Grignon et al. (16) recently demonstrated in stage I-III patients that clinical stage, tumor ploidy and the mitotic rate were independent parameters predicting the outcome. Analysis of nuclear grade did not add any information concerning the prognosis (16). In our study, which also included patients in stage IV, clinical stage and level of pseudouridine excretion were independent prognostic factors. No further prognostic information was added by analysis of grade, tumor size, age, or sex. It seems reasonable to expect that an increase of the proliferating cell fraction would lead to higher transcriptional activity and hence to an increase in the turnover of RNA.

In summary we conclude that analysis of pseudouridine excretion can lead to a better understanding of the natural history of renal cell carcinoma. It may also be an aid in the evaluation of the efficacy of different treatment methods.

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