Urol Res (1982) 10:71–74

Urological Research

Nucleic Acid Metabolism of Bladder Carcinoma Cells in Vitro

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Accepted: January 18, 1982

Summary. Nucleic acid metabolism was investigated to determine the metabolic activity of bladder carcinoma cells. Surgically obtained specimens were subjected to this investigation. Radio-active nucleic acid precursors, ^{14}C-Formate via the de novo synthetic pathway, and ^{14}C-Adenine via the salvage pathway, were used. Activities of bladder carcinoma cells were determined by their incorporation rates. The results were as follows: ^{14}C-Formate incorporation was much higher in grade III bladder carcinoma cells than in the normal epithelium of the bladder; it was highest in stage B_2 bladder carcinoma cells. ^{14}C-Adenine was found to be incorporated into nucleic acid bases of bladder carcinoma cells. It was observed that as the grades and stages progressed, higher incorporation rates were observed. Comparison between the activities of de novo synthesis and salvage pathway was made. The latter was more active than the former in bladder carcinoma cells.

Key words: Bladder carcinoma, Nucleic Acids, De novo synthesis, Salvage pathway, Incorporation rate.

Introduction

It has been suggested that malignant growths from bladder mucosa are caused by sudden mutation of genes in the chromosomes. In addition, growths of this malignancy are supposed to be under the control of these genes, which are composed of nucleic acids [4]. To study the metabolism of nucleic acids in this malignancy might be expected to clarify the mechanism of growth.

There is much evidence that nucleic acids are connected with the genetic function of the chromosomes [1, 9]. Nucleic acids are divided into two parts: one is deoxyribonucleic acid (DNA) and the other is RNA (ribonucleic acid). DNA is a main component of genes, while RNA plays a major role for the transcription and translation of the messages from DNA [6]. Cells can divide and grow by the interaction of these two components. In bladder carcinoma a similar process of cell division growth occurs. To examine how these processes occur in this malignany, nucleic acid metabolism was investigated to determine the metabolic activity of bladder carcinoma cells with the use of radio-active nucleic acids through the de novo synthetic pathway, and ^{14}C-Adenine via the salvage pathway.

This paper presents the activities of nucleic acid metabolism of bladder carcinoma cells in vitro.

Patients and Methods

Patients

Twenty-seven patients, who presented between April, 1972 and March, 1978 were included in this study. Twenty-two patients were male and the remaining five female. The average age of the patients was 60.1 years, ranging from 40 to 81. Histological confirmation and grade of the carcinoma cells were classified according to the criteria of WHO [12]. Stages were determined as to Jewett-Marshall's criteria [7, 8].

Five patients belonged to grade 1, 10 to grade 2, and 12 to grade 3. Six patients were defined as stage A, 10 as stage B_1, 10 as stage B_2 and 1 as stage C.

Normal bladder mucosa from five cytectomised patients with bladder carcinoma was used as control.

Methods

1 to 1.5 g of surgically obtained bladder carcinoma tissues were washed three times with Robinson's buffer. Cell separation was done by simple trypsinisation. Measurement of the incorporoation rates was done by Davidson-Smellie's method described elsewhere [10, 11]. Normal human bladder epithelium was used as control. Outline of the procedure was as follows. Bladder cancer cells in 3 ml of Robinson's buffer (pH 7.2) were incubated with 30 μc of ^{14}C-Formate (relative specific activity 3.95 mc/m mole. Daiichi Chemicals, Tokyo) or ^{14}C-Adenine (relative specific activity 5.32 mc/m

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0300-5623/82/0010/0071/$01.00

Table 1. Incorporation rates of ^{14}C-Formate into nucleic acid bases of bladder carcinoma cells in various grades

Grade	*N*	DNA			RNA	
		Adenine	Guanine	Thymine[a]	Adenine	Guanine
Control	5	1.428 ± 0.068	1.383 ± 0.047	3.271 ± 0.092	1.298 ± 0.048	1.316 ± 0.036
Grade 1	5	1.426 ± 0.023	1.410 ± 0.047	3.360 ± 0.097	1.400 ± 0.067	1.402 ± 0.082
Grade 2	10	1.542 ± 0.153	1.565 ± 0.164	3.996 ± 0.277	1.681 ± 0.134	1.593 ± 0.209
Grade 3[b]	12	1.859 ± 0.106	1.841 ± 0.112	4.441 ± 0.354	1.980 ± 0.151	1.958 ± 0.136

Incorporation rates were expressed as relative specific activities x 10^3. (Mean ± SE)
[a] Incorporation rates of DNA-thymine in various grades were highest among those of the bases ($p < 0.01$)
[b] Incorporation rates of each base in grade 3 showed higher values compared with those of the control, grade 1, and grade 2 ($p < 0.01$)

Table 2. Incorporation rates of ^{14}C-Formate into nucleic acid bases of bladder carcinoma cells in various stages

Stage	*N*	DNA			RNA	
		Adenine	Guanine	Thymine[a]	Adenine	Guanine
Control	5	1.428 ± 0.068	1.338 ± 0.056	3.268 ± 0.092	1.394 ± 0.046	1.395 ± 0.041
Stage A	6	1.423 ± 0.022	1.413 ± 0.044	3.515 ± 0.359	1.427 ± 0.085	1.430 ± 0.097
Stage B_1	10	1.600 ± 0.167	1.626 ± 0.171	3.959 ± 0.225	1.768 ± 0.186	1.684 ± 0.321
Stage B_2[b]	10	1.989 ± 0.312	1.942 ± 0.291	4.713 ± 0.454	2.138 ± 0.310	2.129 ± 0.315
Stage C	1	1.930	1.950	4.880	2.050	1.990

Incorporation rates were expressed as relative specific activities x 10^3. (Mean ± SE)
[a] Incorporation rates of DNA-thymine in various stages were highest among those of the bases ($p < 0.01$)
[b] Incorporation rates of each base in stage B_2 showed the highest values compared with the control and stage A ($p < 0.01$)

mole. Daiichi Chemicals, Tokyo) at 37 °C under an atmosphere of 95% oxygen and 5% CO_2. After 3 h incubation the reaction was stopped by addition of 9 ml of 0.6 N perchloric acid. Extraction and separation of each nucleic acid was done routinely. Separation of bases in nucleic acids was performed according to Bendich's method [3]. Specific and relative specific activities were calculated according to the following formulas:

$$\text{Specific Activity (S.A.)} = \frac{\text{c.p.m. of nucleic acid bases}}{\text{mole of nucleic acid bases}} \times 10^3$$

$$\text{Relative Specific Acitivity (R.S.A.)} = \frac{\text{S.A. of nucleic acid bases}}{\text{S.A. of the added precursors}} \times 10^3$$

Incorporation rates were expressed as R.S.A.

To examine which metabolic pathway was much more active, the ratio (^{14}C-Adenine incorporation rates/^{14}C-Formate incorporation rates, S/N) was calculated. The S/N ratio expresses the relative metabolic activity between de novo nucleic acid synthetic pathway and the salvage pathway in bladder carcinoma cells.

Results

Incorporation rates of ^{14}C-Formate into nucleic acid bases of bladder carcinoma cells in various grades are shown in Table 1. In normal bladder mucosa incorporation rates among purine bases did not differ much, while in DNA the thymine incorporation rate observed was much higher ($p < 0.01$). This was the characteristic feature of the ^{14}C-Formate incorporation. Grade I and II incorporation rates were the same for each purine base. There were no statistically significant differences between normal epithelium and grade I and II bladder carcinoma cells. In grade III bladder carcinoma cells the incorporation rate was observed to be much higher than in normal epithelium and grade I and II bladder carcinoma cells ($p < 0.01$).

The incorporation rates of ^{14}C-Formate in various stages were investigated (Table 2). In stage A no statistical differences were observed in comparison with normal bladder epithelium. In stage B_1 the incorporation rates into DNA thymine, RNA adenine and guanine where shown to be higher than normal. In stage B_2 a higher incorporation rate was observed for each base. No conclusion could be reached in stage C due to the small number of cases.

^{14}C-Adenine incorporation rates into nucleic acid bases of bladder carcinoma cells are shown in Table 3. It was clearly seen that the incorporation rates of bladder carcinoma cells were much higher than those of the normal bladder epithelium ($p < 0.01$). Among the grades it was observed that the higher incorporation rates were observed in each base as the grade progressed ($p < 0.01$). The same tendency was also seen in various stages of bladder carcinoma as

Table 3. Incorporation rates of ^{14}C-8-Adenine into nucleic acid bases of bladder carcinoma cells in various grades

Grade	N	DNA		RNA	
		Adenine	Guanine	Adenine	Guanine
Control	5	1.340 ± 0.041	1.329 ± 0.063	1.298 ± 0.016	1.383 ± 0.044
Grade 1	5	5.370 ± 0.092	5.362 ± 0.057	4.954 ± 0.080	5.010 ± 0.180
Grade 2	10	6.378 ± 0.220	6.342 ± 0.200	6.209 ± 0.435	6.147 ± 0.231
Grade 3	12	7.129 ± 0.361	7.251 ± 0.392	6.815 ± 0.461	6.815 ± 0.410

Incorporation rates were expressed as relative specific activities x 10^3. (Mean ± SE)
Bladder carcinoma cells in each grade showed higher incorporation rates than normal bladder epithelium ($p < 0.01$)

Table 4. Incorporation rates of ^{14}C-8-Adenine into nucleic acid bases of bladder carcinoma cells in various stages

Stage	N	DNA		RNA	
		Adenine	Guanine	Adenine	Guanine
Control	5	1.293 ± 0.041	1.301 ± 0.062	1.295 ± 0.015	1.301 ± 0.043
Stage A	6	5.627 ± 0.581	5.610 ± 0.560	5.127 ± 0.569	5.177 ± 0.407
Stage B_1	10	6.685 ± 0.568	6.446 ± 0.479	6.667 ± 0.553	6.403 ± 0.476
Stage B_2	10	6.947 ± 0.441	6.982 ± 0.772	6.794 ± 0.667	6.813 ± 0.710
Stage C	1	6.500	6.590	6.310	6.290

Incorporation rates were expressed as relative specific activities x 10^3. (Mean ± SE)
Bladder carcinoma cells in each stage showed higher incorporation rates than normal bladder epithelium ($p < 0.01$)

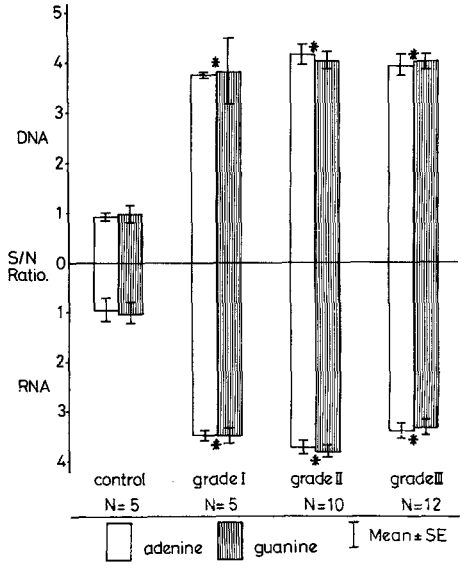

Fig. 1. Comparison of the incorporation activity between de novo synthetic and salvage pathways in various grades: S represents salvage pathway, and N de novo synthetic pathway; *S/N ratio of the bladder carcinoma cells were much higher than normal bladder epithelium ($p < 0.01$)

shown in Table 4. In stage B_1 bladder carcinoma cells the values were much higher than those in stage A. The same situation was also observed between stage B_1 and B_2.

The activity between de novo synthetic pathway and salvage pathway was examined as S/N ratio:

Grades

S/N ratio of the bladder carcinoma cells was much higher than that of the normal bladder mucosa (Fig. 1) but there were no statistical differences between grades.

Stages

Bladder carcinoma cells showed higher values of S/N ratio than normal bladder epithelium (Fig. 2). As seen in grades, no statistical differences were observed among between stages.

Discussion

Bladder carcinoma cells have many metabolic characteristics. Among these metabolic activities one of the most notable pathways is that of nucleic acid synthesis. From the recent advances in nucleic acid research it is very important to observe the metabolic activities on these synthetic pathways. Nucleic acids are composed of DNA and RNA. Analysis of DNA and RNA in other malignant cells indicates that abnormal patterns exist as the tumours grow [5, 13]. In bladder carcinoma cells there have been a few reports of autoradiography by ^{3}H-Thymidine, which is incorporated through the salvage pathway [2]. By this technique a quantitative analysis could not be obtained because thymine and

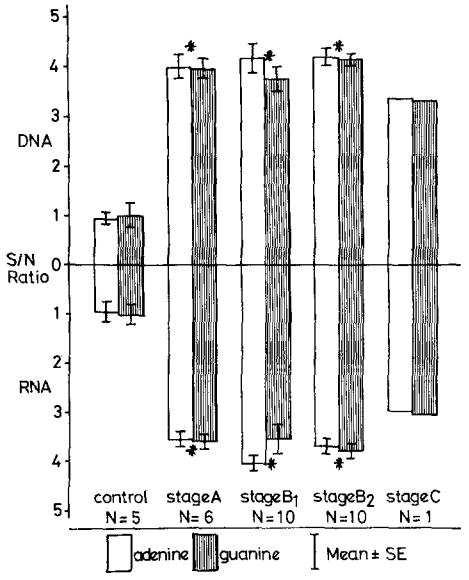

Fig. 2. Comparison of the incorporation activity between de novo synthetic and salvage pathways in various stages: *S/N ratios of the bladder carcinoma cells were much higher than the control ($p < 0.01$)

the thymidine pool in bladder carcinoma cells were not measured. Because of this, investigations were made to examine the activities both in the de novo synthetic pathway by ^{14}C-Formate and in the salvage pathway by ^{14}C-Adenine. ^{14}C-Formate was incorporated into nucleic acids, DNA (Adenine, Guanine and Thymine), and RNA (Adenine and Guanine). On the other hand ^{14}C-Adenine was incorporated via the salvage pathway into nucleic acids, DNA and RNA (Adenine and Guanine).

Incorporation rates of ^{14}C-Formate were observed to be higher in Grade III bladder carcinoma cells. Grade I and II cells were considered to be normal in the de novo synthetic pathway. In Stage B_1 incorporation rates of DNA bases were the same as seen in stage A, but those of RNA showed rather high incorporation rates. These facts indicate that RNA turnover rates in nucleic acid metabolism were greater than those of DNA in this stage. As the stages progressed the incorporation rates become much higher.

It has been postulated that there are many salvage pathways in the nucleic acid metabolism of malignant cells. Experiments with ^{3}H-thymidine indicate the existence of this pathway in bladder carcinoma cells. However, the adenine salvage pathway has not been demonstrated in this malignancy. ^{14}C-Adenine was incorporated into nucleic acids, so that another salvage pathway has been demonstrated in this malignancy. As the grades and stages proceed, higher incorporation rates were observed in the de novo synthetic pathway.

Bladder cancer cells differ from the normal bladder epithelial cells not only in morphology but also in biochemistry. In this study it was demonstrated that malignant cells had great potential for incorporating nucleic acid precursors than normal bladder epithelial cells. These facts led us to consider that malignant bladder cancer cells were undergoing rapid turnover and had much nucleic acid synthesis. According to the cell kinetics there were more members of 4N, 6N, and 8N cells in this malignancy, performing rapid synthesis of nucleic acid [13, 14]. The results obtained in this study also indicate that malignant bladder cancer cells are active in the process of synthesis of nucleic acids, especially through the salvage pathway.

These observations may help in the selection of the most suitable anti-cancer agents in urological practice.

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