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The Effect of Epidermal Growth Factor, Basic Fibroblast Growth Factor, Transforming Growth Factor-β, and Insulin on the DNA Synthesis of Renal Cell Carcinoma Cell Lines.

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SUMMARY : In this experiment, the effect of various growth factors (epidermal growth factor, basic fibroblast growth factor, transforming growth factor-β and insulin) on the DNA synthesis of three renal cell carcinoma cell lines (ACHN, VMRC-RCW, NT) has been investigated in a serum free condition. These growth factors atimulated the DNA synthesis of all renal cell carcinoma cell lines dose-dependently. Transforming growth factor-β, a known growth inhibitor for renal tubular cells, stimulated the DNA synthesis of renal cell carcinoma cells. The conditioned medium (which did not include any serum) contained very litle autocrine growth factor for renal cell carcinoma cell itself. These results suggest that paracrine growth factors are mostly related to the growth of renal cell carcinoma cells than autocrine growth factor. The renal cell carcinoma cells, which are the transformed form of renal tubular cells and due to this transformed character, TGF-β which is basically a growth inhibitor for tubular cell but stimulates the renal cell carcinoma cell.

INTRODUTION

It has been reported that different growth factors are present in the extracts of normal and malignant tissues[1, 2], blood[3] and urine[4]. These growth factors can stimulate or inhibit cellular differentiation as well as they play important role in cellular proliferation. Growth factors have also been identified from conditioned media of hepatoma[5], prostatic cancer[6], colon carcinoma[7] and renal cell carcinoma cell lines[8]. Growth factors stimulate cancer cell proliferation by the autocrine or paracrine manner. It is reported that renal cell carcinoma was originated from renal tubular cells, especially from proximal tubular cells[9]. If we can make a comparative study on the response of different growth factors on renal cell carcinoma cells and normal renal tubular cells, than it will be possible to understand the mechanism of growth of cancer cells. In this experiment, we have investigated the production of autocrine growth factors by renal cell carcinoma cell lines using the serum free culture system and studied the effects of different exogenous growth factors on these cell lines.

MATERIALS AND METHODS

Materials.

EGF was purified from submaxillary glands of male mice using the method described by Savage and Cohen[1]. Bovine basic FGF was obtained from TOYOBO, Osaka, Japan. Insulin was purchased from Sigma, St. Louis, MO. Human TGF-β was from Wako Pure Chemicals, Osaka, Japan. Dulbecco's modified Eagle's

medium was from Nissui Pharmaceuticals, Tokyo, Japan. [^{125}I]-deoxyuridine (2200 Ci/mmol) was from New England Nuclear, Boston, MA.

Renal cell carcinoma cell lines and culture method.

ACHN cells[10] were purchased from Dai Nippon Pharmaceuticals, Osaka, Japan. VMRC-RCW cells were kindly provided by Japanese Cancer Research Resources Bank. NT cells were established in our laboratory[11]. These cell lines were stored in liquid nitrogen. ACHN and NT were maintained in Dulbecco's modified Eagle's medium supplemented with ten per cent FCS. VMRC-RCW cell lines were maintained in DMEM with five per cent non-essential amino-acids and ten per cent FCS. They were incubated in an atmosphere of five per cent CO_2 and 95 per cent air at 37°C and the medium was changed every five days.

Preparetion of the medium conditioned by RCC cell lines.

Each cell line was cultured into 100mm culture dish and when the cells grew confluently, the medium was discarded and the cells were washed. Then fresh serum-free medium was added to the culture and incubated at 37°C for 24 hours. Then the medium was collected and centrifuged at 3000 rpm for ten minutes and stored at -20°C.

Assay of DNA synthesis.

Assay of DNA synthesis of each cell line is shown in Figure 1. RCC cell lines were incubated at 37°C for 24 hours at a density of 5×10^4 cells/cm^2. Then, the medium was discarded and ACHN cells were incubated in the fresh serum-free medium at 37°C for 48 hours; VMRC-RCW and NT cells were incubated in the fresh serum-free medium at 37°C for 24 hours. Then the serum-free medium was renewed and EGF, basic FGF, TGF-β and insulin and stored conditioned medium were added at several concentration. Twenty hours later, one μCi/ml of [^{125}I]-deoxyuridine was added to the cells. Four hours later, the cells were washed and incorporation of [^{125}I] deoxyuridine into the DNA was measured by gamma counter as described before[12].

RESULTS

Figure 2 shows the results of DNA synthesis on RCC cell lines by respective conditioned medium. There was no significant stimulatory effect on DNA synthesis by the conditioned medium. It seems that none of the RCC cell line is secreting autocrine growth factor. EGF, basic FGF, TGF-β and insulin were added to these cell lines and DNA synthesis were studied in order to understand the effect of different exogenous growth factors on RCC cells. Result shows that all the exogenous growth factors stimulate the DNA synthesis of RCC cell lines (**Table 1**). ACHN and NT cells were more strongly stimulated by these growth factors than ten per cent FCS. VMRC-RCW cell was weakly stimurated by these growth factors but was strongly stimulated by serum. Figure 3 A~D show the dose-responce curve of the four growth factors on the DNA synthesis of three RCC cell lines. The maximum stimulation of DNA synthesis was observed by 10ng/ml, 20ng/ml, 10ng/ml, and 10^{-7} mol of EGF, basic FGF, TGF-β and insulin respectively.

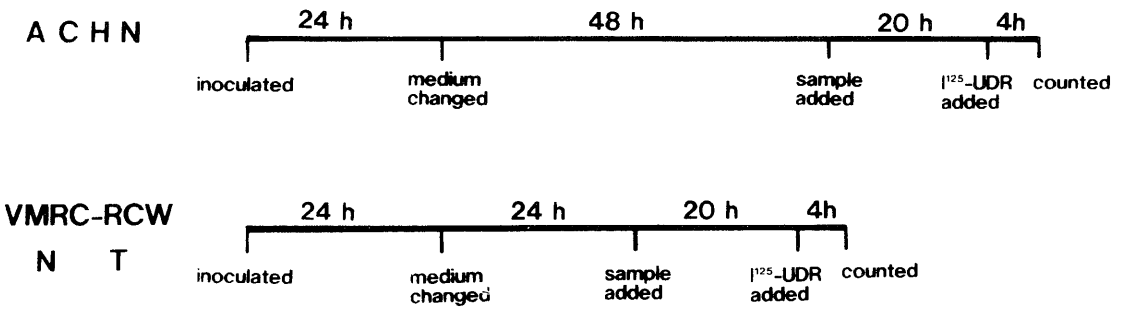

Fig. 1. Time courses of DNA synthesis of renal cell carcinoma cell lines.

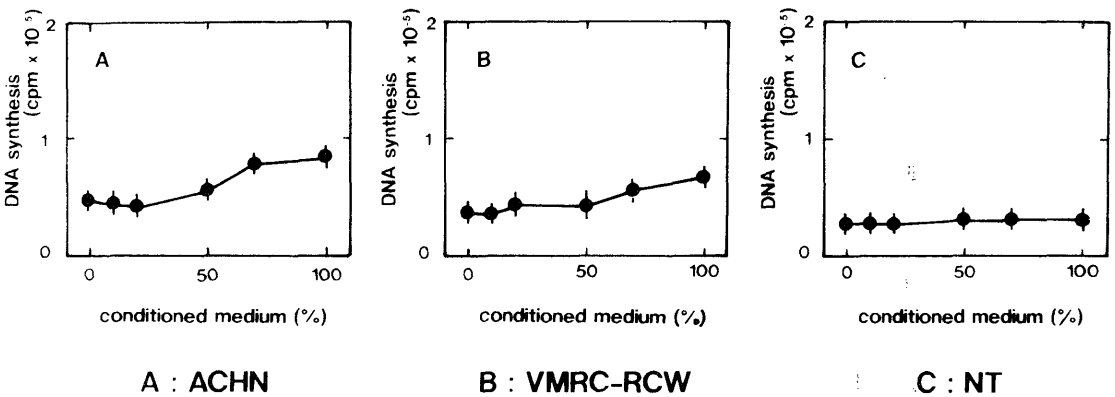

Fig. 2. Effect of the conditioned medium on DNA synthesis of renal cell carcinoma cell lines.

Table 1. Effects of various growth factors on DNA synthesis of renal cell carcinoma cell lines.

addition	DNA synthesis (cpm)		
	ACH-N	VMRC-RCW	NT
none	40493 ± 1124	2617 ± 309	16642 ± 958
EGF 1 ng/ml	79458 ± 1448	3199 ± 988	29372 ± 410
bFGF 2 ng/ml	64090 ± 3657	3556 ± 90	26739 ± 114
TGF-β 1 ng/ml	53767 ± 3859	3186 ± 526	23278 ± 260
insulin 10^{-7} M	49436 ± 127	3421 ± 255	19511 ± 12
10%FCS	50375 ± 2371	11313 ± 657	22607 ± 1039

Experimental conditions were as described in "Materials and Methods". Values are expressed as means ± SD for triplicated experiences.

DISCUSSION

In order to see the effect of different growth factors on RCC cell line, we adopted the DNA synthesis assay method rather than the cell count technique. In the cell count technique, cells may die or detach from the dish that might give false result. In this experiment, we used the serum free culture system, because there are different growth factors present in the serum that might act as a cofactor which may have a direct stimulatory effect to exogenous growth factors.

According to these results all these RCC cell lines were stimulated by exogenous growth factors better than autocrine growth factors under the serum free condition. EGF, IGF-I and FGF have been reported to stimulate the growth and TGF-β has been reported to inhibit the growth of cultured renal tubular cells[12, 13, 14]. But our results showed that TGF-β have weakly stimulated the growth of RCC cells. TGF-β inhibits the DNA synthesis of normal tubular cells, but when these tubular cells are transformed to RCC cells, then this inhibitory effect is altered and TGF-β acts as a stimulatery factor to RCC cells. The same results were obtained when hepatic cells[15] and bronchial epithelial cells[16] were investigated. It is probable that the abolition of inhibitory effect of growth factors to normal cells might allow them to fransform to malignant cells. Our result showed EGF has strongly stimulated the growth of RCC cell. Transforming growth factor-α, which is considered as a transformed type of EGF and acts through the receptor[17] of EGF, stimulates the growth of RCC cells. Renal cell carcinoma is usually a hypervascular tumor. It is probable that FGF stimulates the growth of endothelial

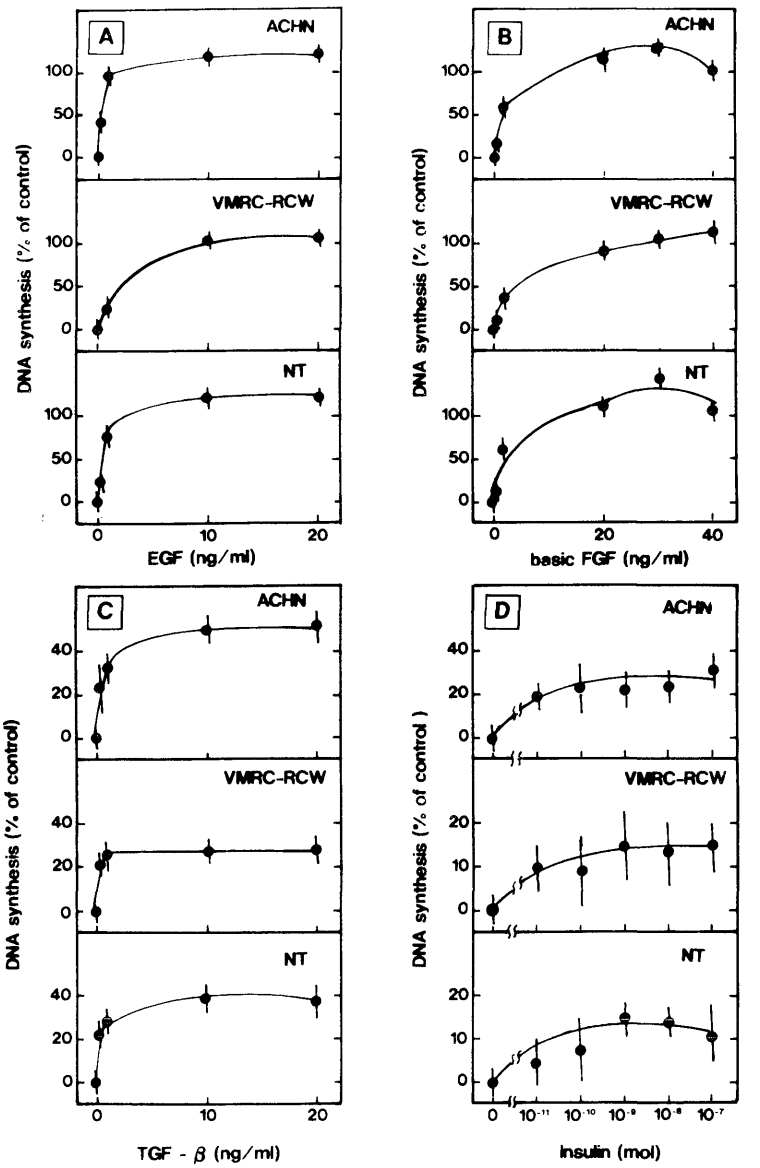

Fig. 3. Dose-responce effect of EGF, basic FGF, TGF-β, and insulin on DNA synthesis of renal cell carcinoma cell lines.

cells and participates in the neovascularization of RCC. FGF also stimurates the growth of RCC cells. Although it was reported that RCC cells produced autocrine growth factors[8, 18]. We could not find the production of autocrine growth factor by RCC cells in our experiment. In the presence of serum, RCC cells produce IL-6 in culture medium[18]. It seems that for the production of autocrine growth factor by RCC cells in culture, same triggers are needed. Further study should be directed to find our the trigger for the production of autocrine growth factors by RCC cells.

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