INFLUENCE OF THE BROWN-PEARCE CARCINOMA GROWTH ON THE CONNECTIVE TISSUE PROLIFERATION

JUN TAKEUCHI

2nd Department of Pathology, Nagoya University School of Medicine
(Director: Prof. Hisashi Tauchi)

It has been established by many workers that the connective tissue has a defensive action against tumor growth. On the other hand, it was reported that formation of young connective tissue around neoplasms may be important in many instances for the growth of malignant cells. Vasiliev,[10] for instance, demonstrated that proliferation of the connective tissue surrounding tumor transplants is favourable for growth and spread of the tumor and that tumor cells have an intrinsic ability to evoke proliferation of connective tissue. Recently, Luciano Ozzello[3] *et al.* described that human breast cancer cells can be maintained in good condition when the medium is supplemented by acid mucopolysaccharides.

Thus, the interaction between tumor growth and connective tissues has still to be made clear. In view of this, influences of tumor growth upon the connective tissue have been studied using the Brown-Pearce carcinoma of the rabbits, and the findings are presented in this paper.

MATERIALS AND METHODS

For the tumor tissue, Brown-Pearce carcinoma, which was proliferated in the testis or thigh muscle of rabbits, was used. The rabbits used in this experiments were male weighing 2–2.5 kg, and have been kept in cages and fed with bean curd and some vegetables.

Polyethylene ring with a mica lid and tube (Fig. 1) was sterilized in invert soap solution and was implanted subcutaneously in the abdominal region of each experimental rabbit. After one week, when the connective tissue was proliferated around the ring, the abdominal skin of rabbits in which the rings were implanted was incised and then the tube installed in the ring was exposed. 0.3 ml of one of following solutions or suspensions was injected through the tube into each ring respectively:

1. Fresh tumor tissue suspended in saline (T).
2. Fresh tumor tissue suspended in 1% chondroitin sulfate solution (TC).
3. Tumor, stored for four days at a temperature of 0°C, suspended in saline (TS).
4. Tumor, stored for four days at a temperature of 0°C, suspended in 1% chondroitin sulfate solution (TSC).

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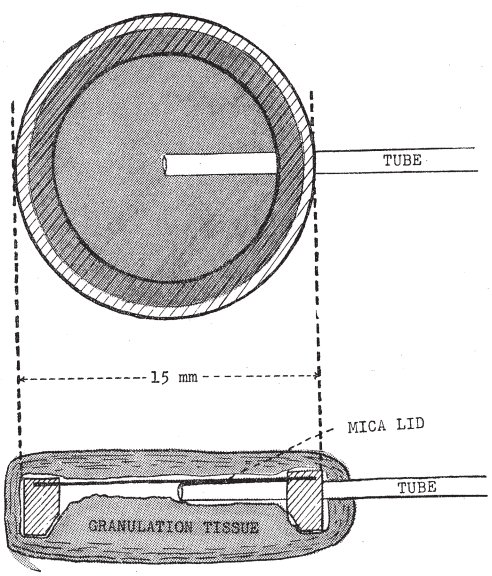

FIG. 1. Polyethylene ring with a polyethylene tube and mica lid.

5. 1% chondroitin sulfate solution (C).

6. Saline (S)..

The suspensions of T, TC, TS and TSC were prepared to contain 0.5 g of tumor tissue per ml.

On the 4, 7 and 10th days of inoculation the animals were sacrificed by air emboli. To prepare the specimens of tissue spreads, the pieces of tissue were taken at a distance of 2 mm from the inner surface of the lid. Spreads were made by Jasswoin's[1] method and stained by PAS, Haematoxylin-Eosin and Jasswoin's[1] method. The other part of the tissue formed in the ring was fixed in 10% formalin solution and embedded in paraffin.

Differential counts of cells in these spreads were made; one thousand cells were counted in each spread, and they were classified according to Vasiliev's[11] description as follows: a) fusiform cells b) mature fibroblasts c) young fibroblasts d) macrophages e) lymphoid cells f) polymorphnuclear leucocytes.

Maximow[2] described the transitional form between young fibroblasts and macrophages. There were also seen fusiform shaped cells with cellular processes but with the nuclei looking like those in macrophages, and they were counted as young fibroblasts in this experiment.

The chondroitin sulfate solution was obtained from Kakenyakukako Co., Tokyo, Japan.

RESULTS

In all cases, the granulation tissues proliferated in the polyethylene rings, and in the groups with T and TC, the tumor cells grew vigorously and filled the ring and in some cases grew infiltratively out of the rings.

TABLE 1. Percentages of Different Cell Forms in the Subcutaneous Granulation Tissues near Polyethylene Ring*

	(1) Fusiform cells	(2) Mature fibroblasts	(3) Young fibroblasts	(4) Macrophages	(5) Lymphoid cells	(6) Polymorph nuclear cells
4 days after injection						
T	5.8±0.1	19.7±1.5	51.0±1.6	12.7±0.9	7.7±0.6	3.1±0.3
TC	4.3±0.3	13.5±1.1	58.9±1.6	16.1±1.2	5.6±0.5	1.6±0.1
TS	3.6±0.2	42.3±4.0	37.7±1.7	12.2±0.9	2.5±0.1	1.7±0.3
TSC	4.7±0.2	35.1±3.7	43.6±2.4	13.6±1.8	2.0±0.2	1.0±0.2
Ch	3.5±0.7	37.9±0.8	15.6±1.2	35.5±2.0	5.9±0.3	1.5±0.1
S	3.7±0.1	49.1±1.7	22.9±1.7	16.7±1.4	5.9±0.1	1.9±0.4
7 days after injection						
T	4.7±0.3	27.4±2.2	39.2±3.8	17.0±2.1	9.2±0.4	2.5±0.4
TC	4.0±0.2	20.0±2.5	39.6±0.7	23.4±1.7	9.6±0.4	3.4±0.5
TS	3.4±0.3	45.4±1.6	32.2±3.0	10.3±1.6	5.4±0.4	3.4±0.2
TSC	3.4±0.3	28.4±1.5	40.8±0.4	21.4±1.8	4.1±0.3	1.9±0.1
Ch	3.5±0.4	30.9±0.8	43.0±1.0	17.7±1.4	3.5±0.5	1.4±0.4
S	2.8±0.5	63.2±5.1	20.2±0.9	10.1±3.0	2.9±0.6	0.8±0.1
10 days after injection						
T	3.9±0.3	23.2±0.7	44.8±2.2	19.1±1.2	4.7±0.4	4.4±0.8
TC	3.5±0.3	20.5±1.3	46.7±3.2	20.5±2.4	6.4±1.2	2.5±0.6
TS	4.0±0.5	46.5±1.0	28.2±1.8	14.2±1.6	4.1±0.4	3.0±0.2
TSC	4.0±0.4	41.8±3.2	28.8±4.6	17.5±0.9	5.2±1.0	2.7±0.4
Ch	3.3±0.2	44.8±1.7	29.0±1.5	15.9±2.1	3.3±0.4	3.7±0.5
S	2.1±0.3	65.4±2.4	21.6±1.7	8.2±1.2	2.2±0.1	0.5±0.1

* Mean percent for each cell form ± standard error is given. Each value indicates average of four rabbits.

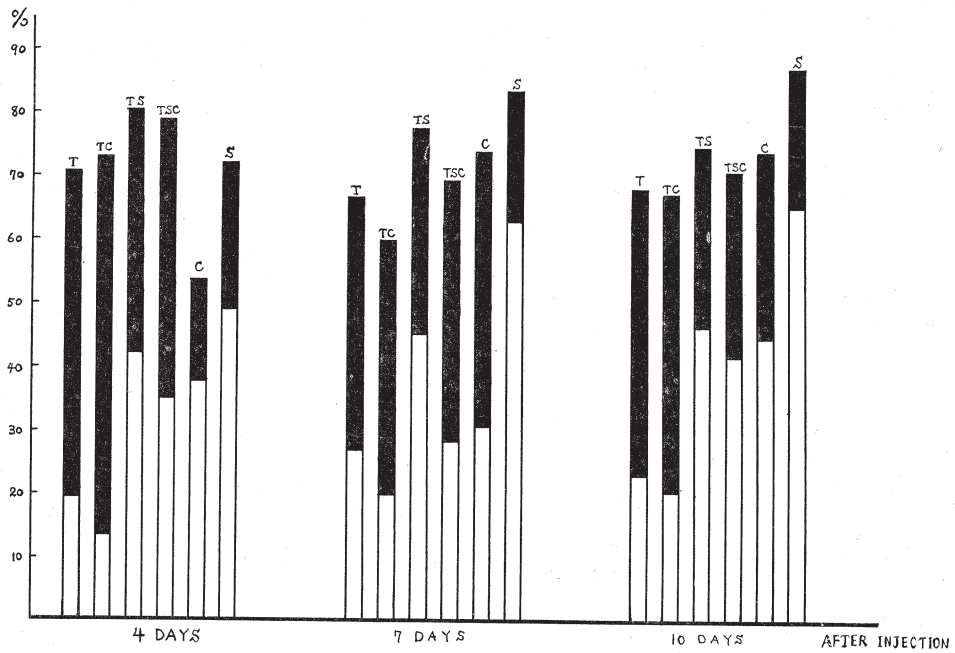

FIG. 2. Percentages of mature fibroblasts and young fibroblasts in the granulation tissue proliferated in the polyethylene ring.
Mature fibroblasts ▬ Young fibroblasts

Changing processes in number of mature and young fibroblasts were indicated in Table 1 and Fig. 2. Throughout this experiment, the granulation tissues with tumor cells in the groups T and TC consisted of a larger number of young fibroblasts and of a smaller number of mature ones, and on the contrary, the number of mature fibroblasts exceeded that of young ones in the other groups. Numerical comparisons of young fibroblasts in the granulation tissue were made between the group C and the group S, and it was noticed that young fibroblasts were larger in number in the group C than in the S, and also larger in number in the group TC than in the T.

DISCUSSION

The present data demonstrated that the granulation tissue around the growing tumor cells consisted of a smaller number of mature fibroblasts and a larger number of young fibroblasts and that this tendency was seen in the cases in which chondroitin sulfate solution was added. It may be considered that the tumor growth promotes the proliferation of connective tissue but inhibits their maturation, and that this influence on the proliferation and maturity of connective tissue depends on the quality and quantity of acid mucopolysaccharides in the tumor tissue.

The present author[4)-9)] previously reported that there was greater quantity of sulfated mucopolysaccharides in the peripheral portion of the vigorously growing tumor tissue and that the connective tissue was composed mainly of young fibroblasts and showed ill-developed fibrous structure.

Therefore it may be considered that the surrounding and cementing intercellular components of the tumor tissue contain greater quantity of sulfated mucopolysaccharide, and that these components promote the proliferation of young fibroblasts and tumor cells and inhibit the maturation of fibroblasts.

SUMMARY

Granulation tissue was made by subcutaneous implantation of polyethylene rings. The Brown-Pearce tumor cells were suspended in saline or chondroitin sulfate solution and each suspension was inoculated into the ring through the installed tube. On the 4, 7 and 10th days of inoculation, the classification and counting the cell types was made on the spreading specimens of granulation tissue that is formed around the transplants in each group. It was found that the granulation tissue proliferated with the tumor cells consisted of a smaller number of mature fibroblasts and a larger number of young fibroblasts. And this tendency was slightly enhanced when chondroitin sulfate was added.

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