SMALL DOSE OF 2-DEOXY-D-GLUCOSE COMPLETELY EXCLUDES THE OVERGROWTH OF FIBROBLASTS

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Synopsis  The present work has been undertaken to assess the effects of a small dose of 2-deoxy-D-glucose on proliferation of fibroblasts in culture by morphological criteria. Fibroblasts in culture medium with 2-deoxy-D-glucose proliferated only to a slight degree, but cell confluence was not achieved. They became swollen and the cytoplasm was increasingly occupied by lucent area. These changes became prominent on the 10th day of incubation. When cell mixtures of fibroblasts and TYK-nu (cell line of undifferentiated adenocarcinoma of the ovary) or TYK-nu only were incubated under the influence of 2-deoxy-D-glucose, the degenerative changes aforementioned were observed only in fibroblasts, while no significant changes were ascertained in the cell line of the cancer by morphological criteria.

These results suggest that a small dose of 2-deoxy-D-glucose shows considerable promise in specific inhibition of fibroblastic proliferation during the process of establishing the cell line of the cancer.

Key words: Fibroblasts • TYK-nu • Mixture of fibroblasts and TYK-nu • 2-deoxy-D-glucose

Introduction

It is not uncommon that overgrowth of fibroblasts is experienced in the process of establishing cell lines of the cancer cells. The excessive growth of fibroblasts occasionally proves to be a hazardous obstacle, though it is quite unfortunate that very few effective approaches have been documented. Recently, it has been reported that either 2-deoxy-D-glucose or 2-deoxy-fluoroglucose is effective in excluding overgrowth of fibroblasts, maintaining the functional properties of the endocrine cells. Therefore, in the present work, low dosis of 2-deoxy-D-glucose was applied to prevent fibroblastic proliferation and the usefulness of this agent was assessed by morphological criteria.

Materials and Methods

Fibroblasts were obtained from the normal lung tissue on occasion of surgical treatment. Established cell line of undifferentiated adenocarcinoma (TYK-nu) was generously donated by Dr. N. Yoshiya.

In the present study, four sets of cells were prepared, i.e. a) fibroblasts (as control), b) fibroblasts, c) mixture of TYK-nu and fibroblasts and d) TYK-nu. Cell set c and d were prepared to investigate whether 2-deoxy-D-glucose did affect the proliferation of cancer cells per se. Two kinds of culture medium were prepared. One consisted of F-10 (GIBCO, Grand Island, New York) supplemented with 20% heat-inactivated fetal bovine serum (GIBCO, Grand Island, New York) and kanamycin sulfate at a concentration of 80μg/ml (Medium A) and the other was composed of Medium A to which was added 2-deoxy-D-glucose (1mM; Medium B) as previously reported. Control fibroblasts were incubated in Medium A and the other three sets of cells were incubated in Medium B following 48 hour pre-incubation after the initiation of culture. Both Medium A and Medium B were renewed every 48 hours. At the 2nd (as control day) and 10th day of incubation, effect of 2-deoxy-D-glucose was assessed by morphological criteria.

Results

a) Control fibroblasts (Fig. 1a, b): Fibroblasts in Medium A proliferated excessively as incubation time advanced. On the 10th day, complete confluence was achieved, when fibroblasts in dishes
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extended their processes and assumed typical wavery and textile appearance.

b) Fibroblasts (Fig. 2a, b): Fibroblasts in Medium B proliferated to a slight degree. Cell confluence was not achieved as shown in Fig. 2b. As early as on the 4th day of incubation, most of the fibroblasts became swollen and lucent area increased in the cytoplasm. These changes proved to be most prominent on the 10th day. It was shown that cells obtained at this stage did not possess the capacity further to proliferate in the renewed dishes containing Medium A. From these results, it is considered that at least two courses of 2-deoxy-D-glucose treatment are needed to achieve complete exclusion of the fibroblasts with the initial cell density as shown in Fig. 2a.

c) TYK-nu and fibroblasts (Fig. 3a, b): A cell mixture of TYK-nu and fibroblasts were incubated in Medium B with cell proportion as shown in Fig. 3a. On the 10th day of incubation, both viable cancer cells and swollen, partially disrupted fibroblasts were observed. Density of the viable cancer cells was not significantly different from that of Set d, while some of the cancer cells revealed slight to moderate degenerative changes.

d) TYK-nu (Fig. 4a, b): TYK-nu in Medium B underwent slight degenerative changes, but cell confluence was almost achieved on the final day of
Fig. 3a. This microphotograph shows a cell proportion of TYK-nu cells and fibroblasts on the control day (original magnification; ×16).

Fig. 3b. A cell mixture of TYK-nu cells and fibroblasts on the 10th day of incubation is shown. The number of fibroblasts, swollen and partially disrupted, is very small. Some of the cancer cells underwent slight to moderate degenerative changes, however, cell confluence was almost achieved and cell density is not significantly different from that of Fig. 4b (original magnification; ×100).

Discussion

Many oncologists have been engaged in establishing cell lines of the cancer cells on research bases. However, it is not uncommon to experience overgrowth of fibroblasts, leading to the inhibition of further proliferation of the cancer cells per se. Several additional approaches have been desired to reduce the number of fibroblasts in culture.289

Recently, Yoshida and his collaborators41–71 demonstrated that 2-deoxy-D-glucose is effective for excluding overgrowth of fibroblasts. One possible explanation for the beneficial effects of 2-deoxy-D-glucose in selective deletion of fibroblasts has been proposed, explaining that a long-term exposure to 2-deoxy-D-glucose affects glycolysis in fibroblasts. 2-deoxy-glucose-6-phosphate, which is converted metabolite from 2-deoxy-D-glucose, has been shown to inhibit the activities of phosphohexoisomerase and glucose-6-phosphate dehydrogenase. Fibroblasts under an ordinary state may produce a poor nutritional condition by quantitative consumption of glucose and other nutrients. This may suggest that fibroblasts under 2-deoxy-D-glucose treatment take up both glucose and 2-deoxy-D-glucose in a
mole to mole manner, leading to an absolutely glucose deficient state which would be an unfavorable situation for fibroblasts in terms of glycolysis or glycoprotein synthesis. At the present time, it remains uncertain whether a selective deletion of fibroblasts arises from specific inhibition of glycolysis in fibroblasts. Thus, the molecular details of 2-deoxy-D-glucose and the further significance of the carbohydrate metabolism in fibroblasts should be strongly investigated.

In any way, addition of 2-deoxy-D-glucose at a low concentration was proved to be an effective method for reducing the number of fibroblasts in culture and might be promising for establishing cell lines of the cancer.

References

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