Dynamic Movement of Hemicysts Reconstructed from Monolayer Cultures of Rat Thyroid Cells in Isologous Serum

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GOTO, K., HOSAKA, S., ISHII, H. and NAGURA, H. *Dynamic Movement of Hemicyst Reconstructed from Monolayer Cultures of Rat Thyroid Cells in Isologous Serum.* Tohoku J. Exp. Med., 1991, **164** (4), 231-236 — Time-lapse studies using cine-microscopic observation showed that the hemicysts formed in a culture of rat thyroid cells in a high concentration of isologous serum were found to arise by swelling from the monolayer sheet, gradually increase in size, and then rapidly collapse. The hemicyst volume increased linearly. We speculate that each hemicyst epithelium transport a constant volume of liquid into the hemicyst lumen. ——— hemicyst; monolayer culture; rat; thyroid gland; isologous serum

Reorganization of monolayer cultures of isolated sheep thyroid epithelial cells is induced by the addition of thyrotropic hormone (TSH) (Kerkof et al. 1964). The hormone dependency of reorganization has been investigated in detail (Fayet and Lissitzky 1970; Fayet et al. 1971). Spinner culture of isolated rat thyroid cells formed multicellular aggregation and the addition of TSH induced follicle-like structures (Inoue et al. 1980).

Previously we reported that a high concentration of isologous serum effectively maintains mucus producing ability of rat colo-rectal adenocarcinoma (Goto et al. 1980). Then we applied this method to rat thyroid cell cultures. A number of large, three-dimensional (about 200 μ m in diameter) hemicysts are formed, when dispersed thyroid epithelial cells from ACI/N rats was cultured in a medium supplemented with isologous and high-concentration serum (Goto 1978; Goto et al. 1991). Electron microscopic studies showed that each hemicyst

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consists of a monolayer of thyroidal epithelial cells and that the microvilli of hemicysts are facing toward the outside, opposite to the lumen, i.e., the polarity of the epithelial cells of hemicysts is opposite to that of normal thyroid follicles (Goto 1978).

In the present study, we use the time lapse cinematographical technique to analyze the mechanism for the formation of hemicysts in thyroid cell monolayer cultures.

MATERIALS AND METHODS

Thyroid glands from 20 ACI/N rats weighing about 250 g were used (Goto et al. 1975). The epithelial cells were dissociated as described in forgoing papers (Goto et al. 1991) and suspended in 2 ml of Eagle's MEM (Gibco, Grand Island, NY, USA) supplemented with 2 mM L-glutamine, 0.2 mM serine, and 2 mM pyruvate, and then explanted into 30-mm plastic Petri dishes (Falcon, Lincoln Park, NJ, USA). This medium was enriched with 40% ACI/N rat serum. The cultures were maintained at 37°C in an atmosphere of 95% air and 5% CO₂ with high humidity.

For time lapse cinematography the filming interval was 1 frame per 2 min. Electric pressure was 3.8 V at a magnification of 50 times. Exposure time was 0.5 sec. The cultured plastic dish was incubated in a large Petri dish, set on the stage of an inverted microscope, in which the mixture of 5% CO₂ and 95% air circulated at 37°C. The size of the hemicysts observed by cinematography was analyzed with a motion analyzer.

The size of a hemicyst was measured: a = the surface average diameter of a hemicyst in contact with the plastic dish, b = the longest average diameter of a hemicyst, when the hemicyst was larger than a hemisphere. A hemicyst formed in a culture medium containing 40% ACI/N rat serum was nearly spherical, and could be considered as a hemisphere when the hemicyst was very small. The volume of the hemicysts was calculated by the following equations:

in the case where $b > a$, $V = (4/3)\pi(1/2)^3 - (2/3)\pi(a/2)^3 + \pi/3(a/2)^2 - (b/2)^2 (2(a/2)^2 + (b/2)^2)$;

in the case where $b = a$, $V = (2/3)\pi(a/2)^3$.

RESULTS

After the addition of the isologous high-concentration serum to the medium, the cultured thyroid cells first formed a monolayer, swelled as a circular form, and then transformed to a hemicyst (Fig. 1a and b). The gradual increase of the size, the hemicyst collapsed. For the observation of the dynamic movement thereafter, the process of hemicyst formation was investigated by the time lapse cinematography (Fig. 2). The isolated thyroid cells were extended and changed to hexagonal, monolayer form. Then hemicysts were reorganized. They were gradually increased in their sizes, and shrunk within short time. The analysis of the motion analyzer (Fig. 3) showed that the movements of the hemicysts were not synchronized with one another, but were at random. One cycle of swell and collapse took a short time range being from 1 to 10 hr.

DISCUSSION

Time-lapse study with a cine microscopic device demonstrated that the

Fig. 1. Phase-contrast micrographic feature of thyroid cells cultured with 40% ACI/N rat serum for 7 days. (a) Focus is in the monolayer cells; (b) in the top of hemicysts. Bars = 100 μ m.

hemicysts were not static structures, but exhibited a dynamic movement, that is, swelling, collapsing, and again swelling. They swelled gradually, and then shrunk rapidly.

The mechanisms of the dynamic action of hemicysts was speculated as followed: The cultured thyroidal cells form a monolayer (Fig. 4a). Water and other component of the medium are transported from the medium into the potential space between the sheet of cells and the glass surface (Fig. 4b). Fluid is driven into the plane of cleavage between the epithelium and glass, resulting in hemicyst formation (Fig. 4c). However, as the hemicyst wall becomes distended by the fluid accumulation, opening between the intercellular spaces with tight junctions or damage in the epithelial membrane appears (Fig. 4d), and the fluid is

Fig. 2. Time lapse cinematography. The various sizes of hemicysts gradually increased in size, and rapidly collapsed. The large hemicyst in the upper portion (a) arose from the monolayer, (b) became bigger, (c) grew up to biggest hemicyst, and (d) collapsed abruptly. The arrows indicate the uppermost lateral margin of the large hemicyst.

released into the medium as the hemicyst collapses (Fig. 4e).

Two lines of evidence are consistent with this interpretation (Goto et al. 1991). The outer surface of hemicysts was equipped tight junctions as shown in our previous studies (Goto. 1978), and the fluid in the medium is thought to be actively transported through the cells. However, the dense epithelial cell

Fig. 3. Changes of the hemicyst volume measured from cine time-lapse studies.

Fig. 4. A proposed mechanism of the movement of hemicysts. The explanation is described in the discussion.

monolayer growing in the surface of a filter membrane — the outer surface of this monolayer was equipped tight junctions — did not give rise to hemicysts. Presumably, fluid in the hemicyst is transfused through the filter membrane and not accumulated in a cavity between cell monolayer sheet and a pore-having filter membrane. The inhibition of the hemicyst formation in a plastic dish by ouabain also conformed to this interpretation. We have found that the addition of ouabain markedly reduced the number of hemicysts formed in isologous serum (Goto et al. 1991). The blocking by ouabain of the transport of fluid from one side of the epithelial sheet to the other is best interpreted as the inhibition of an ATPase activity which is essential for pinocytosis by the plasma membrane of culture cells (Nagura and Asai 1976).

Expansive and contractile movements of thyroidal hemicysts is important to understand the mechanism of, for example, the morphological change of thyroid follicle by TSH, and these hemicysts may be a useful tool for studying thyroidal

physiology and biochemistry.

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