Effect of Homologous High-Concentration Serum on the Mucus Production of Transplantable Colo-Rectal Adenocarcinoma in Culture

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GOTO, K., KUROKAWA, Y. and SATO, H. Effect of Homologous High-Concentration Serum on the Mucus Production of Transplantable Colo-Rectal Adenocarcinoma in Culture. Tohoku J. exp. Med., 1980, 130 (3), 309-310 — Homologous high concentration serum was used for a culture of mucus producing transplantable adenocarcinoma. The mucus secretion of the tumor was maintained in the homologous high-concentration serum. ———— homologous high-concentration serum; transplantable adenocarcinoma; mucus production; tissue culture

Tissue from a transplantable mucus-secreting adenocarcinoma of the colon and rectum in ACI/N rats (Goto et al. 1975) was removed. Blood vessels and connective tissue were dissected from the tissue, and the parenchyma was cut into approximately 1-mm pieces. These were dissociated with gentle stirring in a solution of Ca++, Mg++ free phosphate buffered solution (PBS) containing 1% trypsin at 37°C for 30 min. The dispersed tissue was washed three times with PBS, then cultured (50-mm plastic Petri dishes) in 4 ml of Eagle’s MEM (Gibco). This medium was enriched with either calf serum, fetal calf serum, horse serum, chicken serum (Flow Laboratories), Donryu rat serum, or ACI/N rat serum. The complement of these sera was inactivated. The cultures were maintained at 37°C in a 5% CO₂ incubator. The medium was changed 3 or 4 times a week. When cell growth reached saturation density, subcultures were prepared using 0.05% trypsin.

The cultured cells in media supplemented with 20% concentration of varying sera exhibited almost the same pattern of cell growth. The cells cultured in more than 30% concentration homologous serum, that is, ACI/N rat serum and Donryu rat serum, did not exhibit inhibition of cell growth, but rather they grew well. The cells cultured in more than 30% concentration heterologous serum showed growth inhibition, demonstrating that in ordinary tissue culture more than 20% of serum concentration cannot be used. Cell clusters were observed in medium supplemented with a homologous serum as shown in Fig. 1. The cell cluster in a radial arrangement composed of epithelial cells was intensively positive of alcian blue and PAS staining (Figs. 2, 3), showing that mucus was produced there. In a culture supplemented with calf serum, cell clusters were observed at primary culture, while cell clusters could hardly be seen after the second passage (Fig. 4). Fig. 5 shows the maintenance effect of various sera on mucus secretion. The cell clusters were maintained in medium supplemented with homologous serum better than with heterologous serum. It was found that the ability for maintenance of mucus secretion rose together with serum concentration.

Serum has been used for the culture of living cells to supplement nutrient medium. Concentrations of heterologous serum greater than 20% have not been used because they induce growth inhibition. We found that, using homologous serum, high concentrations of serum promoted cell growth and maintained the function of mucus secretion. On the other hand, in human pancreatic culture (Goldman and Colle 1976), homologous

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Fig. 1. Cell cluster in medium supplemented with 20% homologous serum. 7th passage. PAS stain ×640.

Fig. 2. Cell cluster in medium supplemented with 40% homologous serum. 3rd passage. Alcian blue stain × 640.

Fig. 3. Cell cluster in 40% homologous serum. 10th passage. PAS stain × 640.

Fig. 4. Multi-layered cells without the formation of cell cluster in medium supplemented with heterologous serum (20% calf serum). Alcian blue stain × 640.

Fig. 5. Effect of serum of varying concentrations on mucus production in cultured R-1 cells.
Calf serum, 10% △; 20% ⊿; ACI/N rat serum, 10% ○; 20% ●; 40% ▼; 60% ▼; 80% ◇; 100% ◆.
About 2 × 10⁴ cells/cm² were seeded in 50-mm dishes. Each point was counted at 10th day. Each point is the mean of 3 dishes.

Low concentration serum has been used as a supplement to medium. Our observations seem to agree to their results on effect of homologous serum concerning the maintenance of structure and function.

Homologous high-concentration serum may be useful for maintaining the mucus secretory function in vitro.

References