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Establishment and characterization of gastric surface mucous cell lines (GSM06 and GSM10) from transgenic mice harboring temperature-sensitive simian virus 40 large T-antigen gene

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In the present study, in order to make an *in vitro* model of gastric mucosa for physiological and pharmacological studies, we established two immortalized gastric surface mucous cell lines (GSM06 and GSM10), which produce periodic acid-Schiff (PAS)- and concanavalin A (Con A)-positive glycoproteins, from a primary culture of gastric fundic mucosal cells of adult transgenic mice harboring a temperature-sensitive simian virus 40 large T-antigen gene [1]. Gastric fundic mucosal cells were isolated as a modification of a previously described method for rats by Schepp et al. [2]. The isolated gastric fundic mucosal cells were cultured in DME/F12 medium supplemented with 2% fetal bovine serum (FBS), 1% ITES (consisting of 2 mg/l insulin, 2 mg/l transferrin, 0.122 mg/l ethanolamine and 0.00914 mg/l sodium selenite) and 10 ng/ml recombinant epidermal growth factor (EGF) in a collagen-coated culture dish. To remove fibroblastic cells from the culture, gastric mucosal cells were incubated in the culture medium containing dispase (25 U/ml) for 24 h. The cells, uncontaminated with fibroblastic cells, were then cloned by colony formation. In our series of three attempts, two cell lines (GSM06 and GSM10) have been established at last. The cells proliferated, attached to the dish and grew until confluent monolayers were formed, and maintained tight contact with neighboring cells. Both GSM06 and GSM10 cells have now been in culture for more than 9 months with regular passaging. The either cell produced

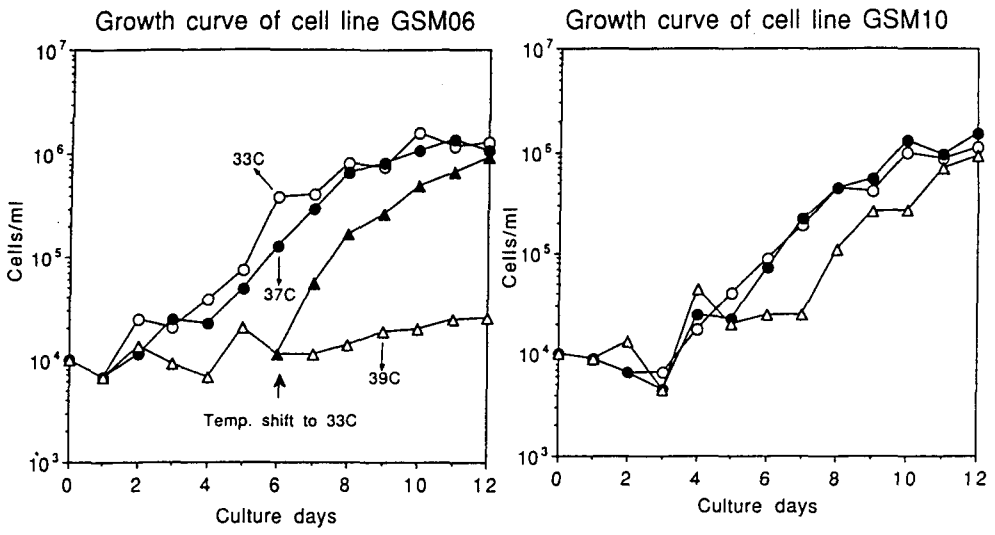

Fig. 1. Cell growth of GSM06 (A) and GSM10 (B) under different temperature conditions. Open circles: at 33℃, closed circles: at 37 ℃, open triangles: at 39 ℃. Closed triangle with an arrow: the temperature was lowered from 39 ℃ to 33 ℃ on day 6. The data represents the mean of results from two dishes.

glycoprotein layers like the gastric surface mucosa in the stomach, and their layers were positively stained by the PAS and class I Con A-horseradish peroxidase (HRP) methods [3]. The glycoprotein layers, however, were not stained by the class III Con A-HRP procedure, which stains only glycoproteins produced by mucous neck cells [3]. In addition, these cells showed neither pepsin activity nor H^+,K^+-ATPase expression (using anti-H^+,K^+-ATPase monoclonal antibody HK4001 [4, 5]). These results strongly suggest that both cells originate from the surface mucous cells, but not from the mucous neck, chief or parietal cells. GSM06 cells exhibited a temperature-sensitive growth in culture (Fig. 1) and expressed T-antigen only at the permissive temperature (33 ℃). In contrast, GSM10 cells displayed the temperature-insensitive growth (Fig. 1) and expressed T-antigen at both permissive and nonpermissive (39℃) temperatures. To our knowledge, this is the first report of the establishment of gastric surface mucous cell lines from animals.

Table 1

Effects of culture periods on production of mucus stained by alcian blue and transepithelial resistance (TER) in GSM06 cells

Culture periods (days)	Mucus (μg alcian blue/cm^2)	TER (Ω/cm^2)
1	20.2 ± 0.84	21 ± 4.6
3	42.6 ± 0.38 *	88 ± 12 *
9	41.3 ± 1.0 *	794 ± 37 *

Data are means ± SEM of 6 experiments. *: $P<0.01$ vs. day 1 (Student's t-test).

In further investigations, we tried to clarify the biological characterization of GSM06 cells. GSM06 cells (1.6×10^5 cells/cm^2) were cultured in Daigo's T medium supplemented with 10% FBS, 1% ITES and 10 ng/ml EGF at 33℃ for 1, 3, or 9 days. Morphological observations revealed that GSM06 cells time-dependently formed microvillus-like projections, and yielded PAS-positive glycoproteins on the cell surface with the growth of the projections. The cells increased the production of alcian blue positive-glycoproteins [6, 7] on days 3 and 9 as compared to day 1 (Table 1). When GSM06 cells were cultured on a membrane filter for 1 to 9 days, elevated transepithelial resistance was noted in a culture-period-dependent fashion (Table 1). In addition, PGE_2 or 6-keto $PGF_{1\alpha}$ production was evoked from the cells from day 1 (Table 2). In order to detect the cell viability, GSM06 cells were labeled with a fluorescence dye, 2',7'-bis(carboxyethyl) carboxyfluorescein (BCECF), which is retained in the intracellular space as long as the cell membrane is intact [8, 9]. Exposure of GSM06 cells to ethanol (7.5%-17.5%) elicited cell injuries in a concentration-related manner on day 1, whereas these cytotoxic effects were attenuated on days 3 and 9 (Fig. 2). This finding

Table 2

Effects of culture periods on *production of prostaglandins* in GSM06 cells

Culture periods (days)	Prostaglandin (ng/10^5 cells/30 min)	
	PGE_2	6-keto PGF_{1a}
1	546 ± 114	15.9 ± 0.95
3	255 ± 29*	16.5 ± 1.1
9	261 ± 32*	28.6 ± 2.1*

Data are means ± SEM of 6 experiments. *: P<0.01 vs. day 1 (Student's t-test).

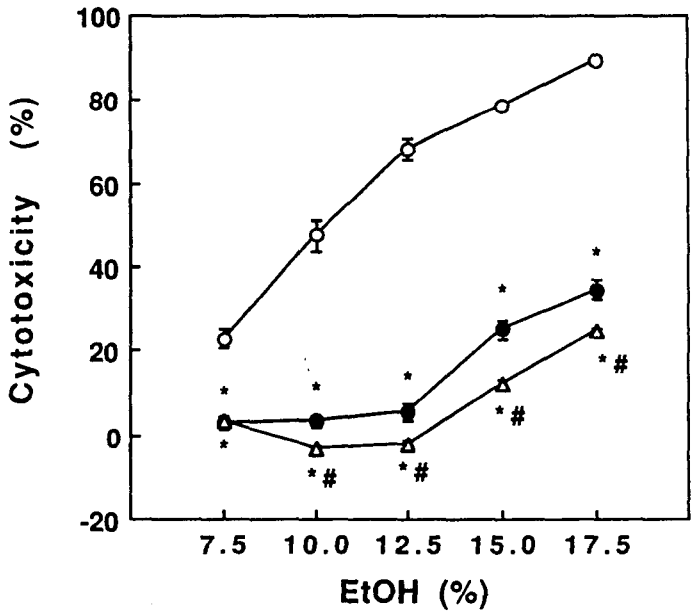

Fig. 2. Effects of culture periods on the cytotoxicity induced by ethanol in GSM06 cells. Open circles, day 1; closed circles, day 3; open triangles, day 9. Each figure represents the means ± SEM for 10 experiments. *: P<0.01 vs. day 1; #: P<0.01 vs. day 3 (Student's t-test).

suggests that these protections may be, at least in part, related to increased glycoproteins and transepithelial resistance.

In conclusion, GSM06 cells possessing unique characteristics should be highly useful as an *in vitro* model of gastric mucosa for physiological and pharmacological investigations. Moreover, experiments using these immortalized cells with normal characteristics established may be able to serve as good substitutes for conventional toxicological studies using living whole animals.

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