ESTABLISHMENT OF RSMG-2 CELL LINE DERIVED FROM MALE RAT SUBMANDIBULAR GLAND IN SERUM-FREE DEFINED CULTURE

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Abstract: We previously established a female rat submandibular gland (SMG)-derived epithelial cell line (RSMG-1) in serum-free defined culture to study the mechanisms of SMG morphogenesis. The SMG has sex dimorphism and associated biochemical manifestations. It is suggested that the male rat SMG-derived epithelial cells may be different in cell biological activities from RSMG-1 cells. Then, we have established an epithelial cell line derived from the male rat SMG, designated as RSMG-2 cell line, and examined the effects of EGF, FGF-1, and TGF-β on the RSMG-2 cell proliferation, compared with RSMG-1 cells.

Key words: salivary gland, serum-free culture, transforming growth factor-β, intercalated duct

Introduction

The submandibular gland (SMG) is a unique organ of which differentiation and development are regulated by sex hormones1-3. The SMG synthesizes and secretes a wide structural and functional variety of polypeptides4, including nerve growth factor (NGF)5, epidermal growth factor (EGF)6, fibroblast growth factor (FGF)-1 and TGF-β1, in particular in males. These products are synthesized by granular duct (GD) cells. In the male SMG, the GD portion predominates whereas in the female, the acinar portion predominates. These findings reveal that the SMG has differences in its function between the male and female, and it is suggested that there may be differences in cell biological characters between the male and female SMG cells. We previously established a female rat submandibular gland (SMG)-derived epithelial cell line (RSMG-1) in serum-free defined medium, which were derived from intercalated ducts (IDs)7. In this study, we have established an epithelial cell line derived from the male rat SMG, designated as RSMG-2 cell line, and examined the effects of EGF, FGF-1, and TGF-β on the RSMG-2 cell proliferation, compared with RSMG-1 cells.

Materials and Methods

Cell culture

RSMG-2 cell line was established as previously described method for RSMG-1 cells8. In brief, SMG excised from 10-week-old Wistar male rats (Japan SLC, Inc., Shizuoka, Japan) were cut, minced, and seeded in 60 mm plastic dishes (Sumitomo Bakelite, Co., Tokyo, Japan) coated with type I collagen (Nitta Gelatin Co., Osaka, Japan). The epithelial cells were maintained in a humidified atmosphere of 5% CO2 at 37°C in MCDB153 medium supplemented with 4 factors (4F) and human recombinant FGF-1 at 1 ng/ml (Upstate Biotechnology, Inc., Lake Placid, NY).
without fibroblast growth\textsuperscript{7-11}). The 4F\textsuperscript{12}) consisted of bovine insulin, 10 µg/ml, human transferrin, 5 mg/ml, 10 µM 2-mercaptoethanol, and 10 µM 2-aminoethanol (all from Sigma, St. Louis, MO). Each factor was prepared as a sterile 100x concentrate and stored at 4°C. To MCDB153 medium ([Ca\textsuperscript{2+}] = 0.03 mM, Functional Peptides Lab., Yamagata, Japan), we added 0.055 g sodium pyruvate (Sigma) and 0.1 g isoleucine (Wako Chemicals Tokyo, Japan) per L. Before reaching confluence, the cells were trypsinized with 0.05% trypsin (Difco Laboratories, Detroit, Michigan) in 0.04% EDTA (Sigma), and then the trypsin was inactivated with 0.1% soya bean trypsin inhibitor (Sigma). The cell numbers were counted with a Coulter particle counter (Beckman Coulter Inc., Fullerton, CA., USA). The cells were reseeded at a split ratio of 1:4. The cell strain, which proliferated continuously for 1 year was termed RSMG-2 cells. RSMG-1 cells used for assay were recovered from the frozen cell stocks which had the similar population doubling levels to the RSMG-2 cells. RSMG-1 cells were cultured also in MCDB 153 supplemented with 4F and human recombinant FGF-1 at 1 ng/ml.

\section*{Results and Discussion}

The male SMG cells began to proliferate between 3 and 10 days after the explant cultures were started from 10-week-old male Wistar rats. Cultures became confluent between 10 and 20 days, and continuous proliferation was maintained for 1 year with subculture every 7 days without undergoing crisis, and the resulting cell line was designated RSMG-2. The cell mor-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Phase-contrast photomicrograph of RSMG-2 cells (A) and RSMG-1 cells (B). Scale bars, 10 \textmu m.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Growth rate of RSMG-2 and RSMG-1 cells. The cell numbers were counted every day in 6-day-culture. The doubling time of RSMG-2 cells (●) was approximately 33 h, and that of RSMG-1 cells (○) was approximately 35 h.}
\end{figure}

\section*{Growth rate}

RSMG-2 and RSMG-1 cells were reseeded in a 24-well plate (Sumitomo) coated with type I collagen at 1x10\textsuperscript{4} cells/well in MCDB153+4F+FGF-1 (1 ng/ml). The cells were counted every day.

\section*{Effects of EGF, FGF-1 and TGF-\beta on RSMG-2 and RSMG-1 cell proliferation}

Before the experiments, RSMG-2 and RSMG-1 cells were cultured in fresh MCDB 153 medium supplemented with the 4F (MCDB153+4F) overnight. The cells were reseeded in a 24-well plate (Sumitomo) coated with type I collagen at 1x10\textsuperscript{4} cells/well in MCDB153+4F. Human recombinant EGF and FGF-1 (Upstate Biotechnology, Inc.) were added to each well at 0-1 ng/ml. The cells were counted after 6 days in culture with a Coulter particle counter.
Male rat SMG-derived cell line

Phylogeny of RSMG-2 cells (Fig. 1A) were the same as the RSMG-1 cells (Fig. 1B). The doubling time of RSMG-2 cells was about 33 h, while that of RSMG-1 cells was about 35 h (Fig. 2). We previously reported that RSMG-1 cells were derived from the ID's. Because we employed the same method which we had established RSMG-1 cells and the morphology of the cells were quite similar, RSMG-2 cells may be derived from the ID's as the RSMG-1 cells. The effects of EGF, FGF-1 and TGF-β on RSMG-2 and RSMG-1 cell proliferation were studied. EGF and FGF-1 individually promoted the proliferation of these cells in a concentration-dependent manner. The effect of EGF on RSMG-2 cell proliferation was not significantly different from that of RSMG-1 cells. FGF-1 was slightly more effective on the RSMG-2 cell proliferation compared with the RSMG-1 cells. On the other hand, TGF-β, which is well known as a differentiation factor, inhibited the proliferation of RSMG-2 cells more than that of RSMG-1 cells. In the SMG, there is a his-

Fig. 3. Effects of EGF, FGF-1, and TGF-β on the proliferation of RSMG-2 and RSMG-1 cells. RSMG-2 (●) and RSMG-1 cells (○) at 1 x 10⁴ cells/well were seeded in a 24-well dish in MCDB 153+4F. (A) EGF was added to each well at 0, 1p, 10p, 100p, 1n, 10n g/ml. (B) FGF-1 was added to each well at 0, 10p, 100p, 1n, 10n, and 100n g/ml. (C) TGF-β was added to each well at 0, 1p, 10p, 100p, 1n, 10n g/ml. The cells were counted after 6 days in culture. Values are the mean±S.E.M. for three measurements.
tological difference based on gender. At birth, the SMG is histologically immature, lacking both definitively acinar cells and granular convoluted tubules (GCT). The ID cells have been thought to differentiate into acinar cells, GCT, or striated duct cells. The sexual dimorphism becomes evident within the gland at 4 weeks of age. In the male SMG, the portion of GCT predominates, suggesting that the ID cells of male SMG differentiate into the GCT more than those of the female, whereas in the female SMG, the portion of acinar cells predominates, suggesting that the ID cells of female SMG differentiate into the acinar cells more than those of the male. These findings indicated that the proliferation ability is not different between RSMG-1 and RMSG-2 cells, but the differentiation ability may be different. It is suggested that the different responses of the male and female SMG cells to the differentiation factor may build the different histological structure of the male and female SMG.

In conclusion, we have established an epithelial cell line (RSMG-2) derived from the male rat SMG in serum-free defined culture. The response of RSMG-2 cells to TGF-β was different from that of RSMG-1 cells although other responses to EGF and FGF-1 are quite similar to the female rat SMG-derived RSMG-1 cells. Thus, these two cell lines may be useful for studying the hormonal regulation of cell differentiation in SMG.

Acknowledgments

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References


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