AN IMMORTAL CELL LINE TO STUDY THE ROLE OF ENDOGENOUS CFTR IN ELECTROLYTE ABSORPTION

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SUMMARY

The intact human reabsorptive sweat duct (RD) has been a reliable model for investigations of the functional role of "endogenous" CFTR (cystic fibrosis transmembrane conductance regulator) in normal and abnormal electrolyte absorptive function. But to overcome the limitations imposed by the use of fresh, intact tissue, we transformed cultured RD cells using the chimeric virus Ad5/SV40 1613 ori-. The resultant cell line, RD2(NL), has remained differentiated forming a polarized epithelium that expressed two fundamental components of absorption, a cAMP activated Cl⁻ conductance (G\textsubscript{Cl}) and an amiloride-sensitive Na⁺ conductance (G\textsubscript{Na}). In the unstimulated state, there was a low level of transport activity; however, addition of forskolin (10⁻⁵ M) significantly increased the Cl⁻ diffusion potential (V") generated by a luminally directed Cl⁻ gradient from -15.3 ± 0.7 mV to -23.9 ± 1.1 mV, n = 39; and decreased the transepithelial resistance (R) from 814.8 ± 56.3 Ω·cm² to 750.5 ± 47.5 Ω·cm², n = 39, (n = number of cultures). cAMP activation, anion selectivity (Cl⁻>I⁻>gluconate), and a dependence upon metabolic energy (metabolic poisoning inhibited G\textsubscript{Cl}), all indicate that the G\textsubscript{Cl} expressed in RD2(NL) is in fact CFTR-G\textsubscript{Cl}. The presence of an apical amiloride-sensitive G\textsubscript{Na} was shown by the amiloride (10⁻⁵ M) inhibition of G\textsubscript{Na} as indicated by a reduction of V" and equivalent short circuit current by 78.0 ± 3.1% and 77.9 ± 2.6%, respectively, and an increase in R, by 7.2 ± 0.8%, n = 36. In conclusion, the RD2(NL) cell line presents the first model system in which CFTR-G\textsubscript{Cl} is expressed in a purely absorptive tissue. It provides an opportunity to study the properties and role of CFTR in the context of absorptive function in immortalized epithelial cells.

Key words: absorptive; cell line; CFTR; epithelium; immortalized; sweat duct.

INTRODUCTION

Cystic fibrosis (CF) is a disease of electrolyte transport that affects both fluid secretion and fluid absorption (37). Nonetheless, even though markedly decreased salt absorption in the sweat duct is a hallmark of CF (18) and Na⁺ absorption is abnormal in the CF lung (30), a cell line has not been developed to study the function and regulation of endogenous CFTR, the Cl⁻ channel protein affected in CF, in a purely absorptive role. Because the function and regulation of CFTR may vary according to the specific purpose of the tissue where it is expressed, a differentiated cell line derived from a purely absorptive epithelium that inherently expresses CFTR will be highly advantageous in defining the role of CFTR in absorption. Cell lines expressing CFTR that are currently available for the study of this protein ex vivo have been derived from either transfected nonepithelial cells, known secretory cells, undefined mixed populations of cells, or if originating from absorptive tissues, fail to express characteristic properties of absorption (1-3,11,13,14,16,17,19-21,24-26,29,31-34,44,47,48).

For this reason, we undertook to transform primary cultures of human reabsorptive sweat duct (RD) cells using a chimeric virus, Ad5/SV40 1613 ori- (45), as a transforming agent. The RD was chosen as a model tissue for several reasons. First, in contrast to other complex epithelia, RD cells are involved in the sole process of NaCl absorption. Secondly, RD cells express high levels of CFTR (more than 80% of the total tissue conductance is due to CFTR; 36,37). Thirdly, it has been shown that RD cells from normal and CF subjects can be grown in primary culture and exhibit certain characteristic electrophysiological properties expressed by the parental tissue (4,8-10,27,28,35,41).

In the present study, we describe the development of a differentiated RD cell line, RD2(NL), which forms a polarized epithelium, expressing two fundamental components of absorption: i) a cAMP activated Cl⁻ conductance (G\textsubscript{Cl}), and ii) an amiloride sensitive Na⁺ conductance (G\textsubscript{Na}). This cell line provides the first opportunity to study the properties of endogenous CFTR in its absorptive role in immortalized cells.

MATERIALS AND METHODS

Culture media. For the initiation and growth of primary duct cultures, and selection of transformed cells the culture medium was MCDB 170 (UCSF Cell Culture Facility, San Francisco, CA) (22), supplemented with bovine pituitary extract (BPE; 70 μg/ml; Hammond Cell Tech, Alameda, CA), epidermal growth factor (EGF; 25 ng/ml), hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (5 μg/ml), ethanolamine (10⁻⁴ M), phosphoethanolamine (10⁻⁴ M), penicillin (50 U/ml), and streptomycin (50 μg/ml). The medium for maintenance of the cell line was a modified keratinocyte (KT) medium (15) comprised of a 50:50 mixture of minimal essential medium α-modification (α-MEM)-Dulbecco’s modified Eagle medium

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Fig. 1. Photomicrograph showing a healthy “island” of epithelial cells growing amidst degenerating and senescing cells in an RD culture 12 d after infection with the chimeric virus. ×300.

Fig. 2. Photomicrograph of the polygonal epithelial cells comprising an RD2(NL) culture at Passage 130, showing the cobblestone appearance typical of epithelial cells in culture. ×300.

(DMEM) (GIBCO, Grand Island, NY), supplemented with 5% Nu serum (Nu) (Collaborative Research, Bedford, MA), EGF (10 ng/ml), hydrocortisone (0.4 μg/ml), transferrin (5 μg/ml), prostaglandin E2 (25 ng/ml), sodium selenite (10-4 M), triiodothyronine (2 × 10-11 M), penicillin (50 U/ml), and streptomycin (50 μg/ml). For initiation of cultures on permeable supports, we used H/D + FBS, a mixture of Ham’s F-12:DMEM (50:50) supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml), and 5% FBS (Intergen, Purchase, NY).

The following day the cultures were fed with one of three different growth media: i) H/D (U-G); a mixture of Ham’s F-12:DMEM (50:50), supplemented with penicillin (50 U/ml), streptomycin (50 μg/ml), and 2% Uroser G serum substitute (U-G) (GIBCO); ii) H/D (BPE + Nu); a mixture of Ham’s F-12:DMEM (50:50), supplemented with BPE (70 μg/ml), 5% Nu, penicillin (50 U/ml), and streptomycin (50 μg/ml); or iii) H/B (BPE + Nu): a modified MCDB 170 medium (Clonetics, San Diego, CA), buffered with HEPES (30 mM) and sodium bicarbonate (24 mM) and supplemented with EGF (25 ng/ml), hydrocortisone (0.5 μg/ml), insulin (5 μg/ml), transferrin (5 μg/ml), BPE (70 μg/ml), 5% Nu, penicillin (50 U/ml), and streptomycin (50 μg/ml). H/D (U-G) was chosen as a growth medium as it had been shown in preliminary experiments to yield cultures expressing the appropriate differentiated properties. H/D (BPE + Nu) was tested to determine the ability of BPE and Nu to replace U-G as a growth supplement, while growth in H/B (BPE + Nu) tested the effect of an enriched medium (MCDB 170), buffered with both HEPES and bicarbonate.

Primary cultures. The skin specimen used for isolation of eccrine sweat glands was obtained from an abdominal incision at surgery after informed consent. The donor was a 34-yr-old female. RD cultures were established according to previously described methods (4,8). Briefly, after digestion in collagenase (85 U/ml) for 24 h, the tissue was transferred to culture medium and the epidermis was pulled from the underlying connective tissue, revealing the RDs that remained attached to the embedded secretory cells (SCs). Further pulling resulted in the separation of the RDs from the SCs at a region near the junction between the two. To be certain that only the RD was selected, this region was then removed and discarded together with a short length of adjacent RD and attached SC. Individual ducts were trans-

| TABLE 1 | BASAL TRANSEPITHELIAL PROPERTIES FOR RD2(NL) CELLS IN DIFFERENT MEDIA* |
|---------|-------------------|-------------------|-------------------|
|         | \( n \) | \( V_l \) | \( R_t \) | \( I_l \) |
| H/D (U-G) | 16 | -1.9 ± 0.3 | 387.0 ± 32.7 | -4.8 ± 0.4 |
| H/D (BPE + Nu) | 11 | -3.2 ± 0.5* | 381.3 ± 35.5 | -8.1 ± 0.8* |
| H/B (BPE + Nu) | 19 | -1.7 ± 0.3 | 455.7 ± 49.5 | -3.8 ± 0.5 |
| Combined | 46 | -2.2 ± 0.2 | 414.0 ± 25.0 | -5.2 ± 0.4 |

* Values are means ± SE. \( n \) refers to the number of cultures examined. \( V_l \), transepithelial potential; \( R_t \), transepithelial resistance; \( I_l \), equivalent short circuit current.

* Significantly different from two other media and combined values (\( P < 0.05 \)).