Strategies to establish a continuous cell line in the shark rectal gland of *Squalus acanthias*

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The rectal gland of the dogfish shark, *Squalus acanthias*, is an extra-renal organ whose purpose is to excrete concentrated sodium chloride from the plasma of the blood into the gut. This specialization includes high concentrations of physiologically relevant membrane proteins that make this gland an ideal model for studying transepithelial chloride transport. Our lab continues efforts to establish a continuous cell line that maintains characteristic transport capabilities.

As the dogfish shark swims through its hypertonic marine environment, the rectal gland acts to rid the blood of excess sodium chloride. This specialized organ is ideal for the study of cystic fibrosis transmembrane conductance regulator (CFTR)-mediated chloride transport across epithelial tissue for several reasons. First, this organ is composed of branched tubules that contain a single cell type specialized for salt transport[1]. Second, these cells express high levels of the membrane proteins Na/K-ATPase, Na/K/2Cl cotransporter, and CFTR[1,4]. Third, secretion from the gland is governed by multiple hormonal and regulatory mechanisms capable of eliciting large (up to 50 fold), sudden increases in sodium chloride transport[1]. Fourth, primary cultures of tubules attain vigorous rates of transport in high resistance monolayers that possess all signal transduction pathways found in the intact gland.[4,5]

In ongoing attempts to establish a continuous cell line from the gland, primary cultures of tubules were prepared and when confluent, cells were transfected with retroviral vector (pQCXIH) plasmids that encode simian virus 40 large T antigen (SV40T) or human telomerase reverse transcriptase (hTERT). Transfection was accomplished using calcium phosphate co-precipitation or polyethylenimine (PEI), and treatments included SV40T and hTERT separately, or in combination. Additionally, an eGFP-expressing plasmid was added to estimate transfection efficiency and the SV40T and hTERT plasmids contained an encephalomyocarditis virus internal ribosome entry site (IRES)-hygromycin B phosphotransferase (IRES-hygR) cassette located downstream of SV40T or hTERT cDNA to enable selection of cells in hygromycin. To each well of a 24-well plate was added 150-600 ng DNA along with 80-90% of input DNA being transforming plasmid(s), of which 10-20% was eGFP-encoding plasmid. Confocal imaging of transfected cells indicated visible but faint eGFP expression was detectable in rare cells. However, upon transfer of primary cultures, the establishment a continuous cell line was not accomplished.

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1. **Forrest JN.** Cellular and molecular biology of chloride secretion in the shark rectal gland: regulation by adenosine receptors. *Kidney Int*. 49:1557-1562, 1996.
2. **Llano M, Saenz DT, Meehan A, Wongthida P, Peretz M, Walker WH, Teo W, Poeschla EM.** An essential role for LEDGF/p75 in HIV integration. *Science*. 314:461-464, 2006.
3. **Silva PJ, Stoff J, Field M, Fine L, Forrest JN, Epstein FH.** Mechanism of active chloride secretion by shark rectal gland: role of Na-K-ATPase in chloride transport. *Am J Physiol*. 233:F298-F306, 1977.
4. **Valentich JD, Forrest JN Jr.** Chloride secretion by primary cultures of shark rectal gland epithelial cells. 1. Characterization of transepithelial transport. *Am J Physiol*. 360:C813-C823, 1991.
5. **Valentich JD.** Primary cultures of shark rectal gland epithelial cells: A model for hormone-sensitive chloride transport. *J. Tiss. Cult. Meth*. 13:149-162, 1991.