UNC
SCHOOL OF MEDICINE
Marsico Lung Institute/CF Center

Novel W1282X Homozygous Nasal Epithelial Cell Line-UNCX2TC

Although the recent development of small molecule cystic fibrosis transmembrane regulator modulator drugs is an exciting advance, they are not approved for individuals with certain mutations, particularly those with premature stop codon class I mutations. Clinical trials of read-through agents have been disappointing, and cell models for these mutations are needed to develop and test potential therapies.

The limited availability of primary airway epithelial cells has driven the creation of cell lines. Bmi-1 is a proto-oncogene that maintains stem cells by suppressing cyclin dependent kinase inhibitors, and its expression creates cell lines that recapitulate normal cell structure and function. In prior studies, the Randell Lab introduced Bmi-1 and the catalytic subunit of telomerase (hTERT) into primary bronchial epithelial cell preparations from three non-CF donors and three homozygous F508del CF donors (Am J Physiol Lung Cell Mol Physiol. 2009 Jan;296(1):L82-91.). Bmi-1/hTERT expression extended cell lifespan in conventional BEGM media while maintaining a diploid karyotype. When seeded at high density on porous supports, passage 15 cells exhibited a pseudostratified morphology with few ciliated cells but abundant mucus secretory cells. Ussing chamber studies demonstrated electrophysiologic properties mostly comparable to early passage primary cells. Like primary cells, Bmi-1/hTERT cell lines had fastidious requirements for growth factors, substratum, adequate seeding density and good husbandry practices.

In more recent experiments, primary homozygous W1282X nasal epithelial cells were obtained and expanded using the “Conditionally Reprogrammed Cell” (CRC) method (a detailed CRC protocol is present in Am J Respir Cell Mol Biol. 2017 May;56(5):568-574.). The CRC passage 1 cells were transduced with a single lentiviral vector expressing both Bmi-1 and hTERT. Unlike the Bmi-1/hTERT transduced bronchial epithelial cells, the nasal epithelial cells did not expand extensively in BEGM media. However, they did expand well using the CRC technique, and cells expanded to passage 15 generated air:liquid interface cultures suitable for Ussing chamber studies (unpublished data).

In view of the pressing need for research and development focused on Class 1 CFTR mutations, the Randell lab will make these cells available pre-publication to all qualified researchers. An MTA is required, and recipient labs should be certified for BSL2 level studies.

A cryopreserved vial will be shipped, and the recipient lab must have vapor phase LN2 storage capabilities. The overall strategy is to grow and expand the cells using the CRC method and to cryopreserve a sufficient quantity of the cells for future studies. Once the cells have been expanded and aliquots are securely stored, they can be used for experiments, including seeding on human type IV collagen coated porous supports for polarized air:liquid interface cultures.

All necessary protocols have been published in detail, and requests for the cells can be directed to Dr. Randell (randell@med.unc.edu).