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Models of ciliary dysfunction: time to expand

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Multiple motile cilia line our airways and beat in a co-ordinated pattern to transport mucus away from the lungs. This mucociliary escalator forms one of the first defences against foreign material entering the lower respiratory tract and mucociliary dysfunction leaves the respiratory tract vulnerable to infection. Ciliary dysfunction has been identified as a feature of several common airway diseases such as asthma, COPD and bronchiectasis, making improvement of ciliary function a tempting target for drug development (1, 2).

Primary ciliary dyskinesia (PCD) is an inherited respiratory condition affecting approximately 1:10,000 of the population (3). Failure of motile cilia to clear secretions from the ears, sinuses and lower airways results in otitis media, rhinitis, wet cough, recurrent upper and lower respiratory tract infections and bronchiectasis (3). Approximately 50% individuals with PCD have complete reversal of left-right symmetry of the internal organs (situs inversus), complex congenital heart disease or other laterality defects due to disrupted ciliary function in the embryonic node. Surprisingly, there are currently no licensed pharmaceutical products directed at correcting ciliary function. One of the obstacles for development of such therapies is the lack of laboratory models to reproducibly study therapeutic effect on a large scale.

Murine models of ciliary dysfunction have been developed but often, due to lack of functioning cilia in the brain, these murine models develop hydrocephalus and die (4, 5). Furthermore mice with mucociliary clearance dysfunction do not develop lung disease which mimics that of humans.

In-vitro air liquid interface (ALI) cultures of differentiated primary epithelial cells are useful models in the diagnosis of PCD (6). ALI cultures, usually grown from nasal brushings, have been shown to reproduce the primary ciliary phenotype in PCD whereas ciliary dyskinesia secondary to bacterial or viral infection normalises (7). However, use of primary ALI cultures at scale presents some difficulties. Firstly facilities are required for growth of human derived samples which are likely to be infected with pathogenic micro-organisms. The largest obstacle is that primary cells can’t typically be expanded beyond 3-5 passages leaving limited numbers of cultures from a single donor. Consequently a large number of different volunteers with ciliary dysfunction willing to have a biopsy or a system to use donor lungs post mortem or post transplant is required to perform experiments at scale. Several methods have been developed to help overcome these difficulties by extending the differentiation potential of primary cells. These methods include, use of a murine fibroblast feeder layer, transformation of cells with BMI-1, and use of ROCK inhibitors to extend proliferation potential of basal cells while maintaining the potential to
differentiate into ciliated epithelial cells (8-10). A well described immortalised cell line which could be reproducibly differentiated at ALI into columnar epithelial cells with dyskinetic cilia would overcome many of these difficulties and allow studies to be scaled up. In the present issue of AJRCMB an article by researchers in Melbourne Australia describe a dyskinetic ciliary phenotype identified in an existing immortalised cell line (BCi-NS1) (11). When cultured at ALI ciliary beat frequency in these cells is lower than controls at \( \sim 4\text{Hz} \) and increases in response to known stimulus (ATP and IL-1\( \alpha \)) demonstrating potential to test modifiers of ciliary beat frequency. The authors describe a circular ciliary beat pattern and central pair agenesis by transmission electron microscopy and propose that this model could be used for screening drugs to enhance ciliary beat frequency and pattern.

Interestingly in primary cells a circular beating of the cilia when visualised from above, is associated with a rare form of PCD accounting for approximately 10% patients with the condition (12). Circling cilia are associated with defects of the central pair projections or the proteins of the radial spoke head such as RSPH4a, RSPH1 and RSPH9 (13). It has been suggested that the electron microscopy phenotype results from a twisting of the central complex and eventual loss of the central pair as a result of the circular beat pattern (14). Cilia in the embryonic node which control right–left symmetry ordinarily lack a central pair of microtubules and beat with a circular beat pattern in health. Resultantly individuals with \textit{RSPH1,4a and 9} genes do not present with situs abnormalities, as nodal cilia are unaffected. A weakness of the study by Kuek et al, is the lack of known mechanism for the ciliary dyskinesia in the BCi-NS1 cell line. The authors have gone to some lengths to try and address this weakness, such as conducting immunofluorescent staining for RSPH1 proteins, and karyotyping with multicolour fluorescence in situ hybridisation for defects in chromosomal patterning. However no clear reason for the ciliary dyskinesia has been established and use of the model will likely be limited by this factor. Despite this, a ciliary dyskinesia cell line could form a useful resource to answer questions about ciliary beat frequency and pattern in rare and common diseases, as well as provide a reproducible model for manipulation of ciliary function. These cells along with alternatives created through emerging iPSc and CRISPR technology could be grown at ALI or as spheroids or organoids to allow an increase in the scale at which studies on ciliary dyskinesia can be reproducibly performed in the future.


