Establishing an immunofluorescence double staining on respiratory epithelial cells for PCD diagnostics

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1. Abstract

The respiratory tract is lined with ciliated epithelial cells that facilitate mucociliary clearance, a critical process for trapping and removing inhaled particles. Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by ciliary dysfunction leading to chronic respiratory symptoms from birth. The axoneme of a cilium consists of nine peripheral microtubule pairs surrounding a central pair, with microtubules formed from tubulin. Various proteins such as radial spokes and dynein arms connect the axonemal components. DNAH5, a part of the outer dynein arms, and GAS8, a part of the nexin-dynein regulatory complex, are essential ciliary proteins that are often affected by mutations causing PCD. This thesis aims to develop a double immunofluorescence staining protocol for DNAH5 and GAS8 proteins in respiratory epithelial cells, which are crucial for the diagnosis of PCD. Initial experiments with a new anti-GAS8 antibody showed promising results, but subsequent testing with patient samples resulted in inconsistent staining intensities that did not meet diagnostic standards. Challenges included potential mucus interference, sub-optimal antibody storage conditions and the use of a paraffin-based liquid marker pen, which can inhibit antibody binding. While the proposed double staining protocol could reduce material usage and analysis time, the inconsistent staining intensity limits its diagnostic utility. Further investigation is required to refine the protocol, including exploring alternative liquid barrier markers and optimizing antibody handling. Until the identified issues are resolved, the original single staining method remains the preferred approach for clinical diagnosis. This work highlights the complexities involved in improving diagnostic methods for genetic disorders such as PCD and the need to optimize technical variables.

2. Introduction

The respiratory tract is lined with ciliated epithelial cells and mucus producing goblet cells. Through ciliary beating the mucus is transported to the pharynx. Inside of each cilium is the axoneme, which consists of nine peripheral microtubule doublets organised around a central pair of microtubules [1]. In Primary ciliary dyskinesia different proteins of the axoneme have structural problems or are missing. This impacts the motility of the cilium and also the mucus transport. Patients suffer from a diverse range of symptoms. Which makes diagnosing it a challenge. A part of the diagnostic toolkit for PCD are immunofluorescence stainings of different axonemal proteins [2]. Some axonemal proteins can be effected by multiple mutations, examples are DNAH5 and GAS8 [3].

In PCD diagnostics these two proteins are stained with a immunofluorescence staining. Originally, for one protein, two slides are needed.

3. Aims and leading questions

The aim of this diploma thesis is to establish a staining protocol for a DNAH5 and GAS8 immunofluorescence double staining on respiratory epithelial cells for PCD diagnostics. To reach this goal we first must establish the staining protocol for a new anti-GAS8 antibody and then combine DNAH5 and GAS8.

Which antibody dilution achieves the best staining based on the following criteria:

- Intensity of the staining
- Background stain

4. Methods and material

Immunofluorescence uses the ability of proteins to function as antigens to specific antibodies. Through antibody-antigen reactions, target proteins can be detected through different systems. In our method we use fluorophore marked secondary antibodies. The secondary antibodies can bind to the target bound primary antibody (Figure 1). The fluorophores re-emit light after excitation of light under the microscope. The emitted light is of a different wavelength as the exciting light.

In our staining protocol we stained two primary antibodies simultaneously.
This is possible, if the two stained antibodies are not derived from the same species. In this case two different secondary antibodies can be used to detect them and be able to distinguish between the two target proteins. Different antibody conzentrations were tested, to reach a good staining outcome.
Staining intensities were rated ,,-´´to ,,+++´´ based on reference pictures.

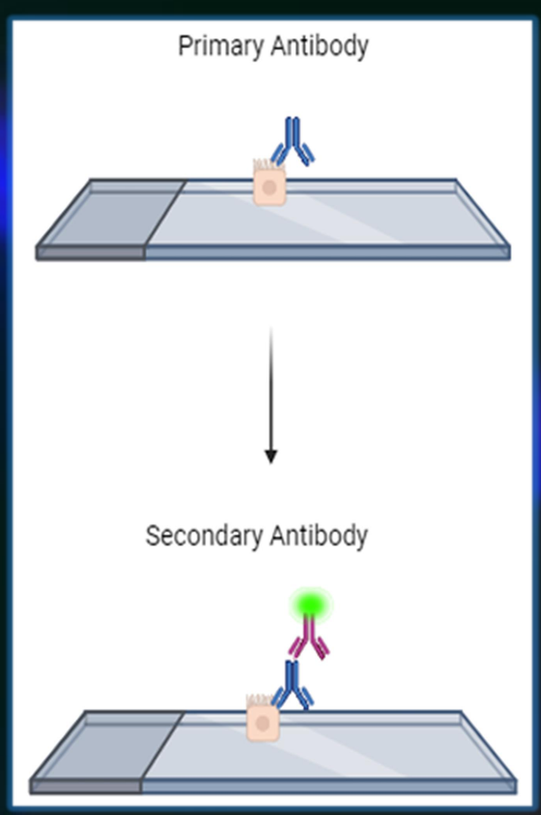


Figure 1 Immunofluorescence

5. Results

We could establish a staining protocol for a double staining of DNAH5 and GAS8. It was possible to use a higher dilution of the antibodies than in the seperate staining protocol used originally. We could reach staining intensities of ,,+++´´ with no background staining. However the staining results varied between staining cycles and between controls and patients.

6. Discussion

As the staining intensities varied between different stainings of the same patients, it might have something to to with the antibody stability when stored after thawing it for the first use. In the patient slides we could not achieve the required intensity in most of the stains, even when we reduced the dilutions. We consider an inhibiting effect to the antibodies of mucus on the cells.

In conclusion, we could not reach the aim of implementing the staining into the diagnostic routine. We established a protocol, but there are still a few open questions.

References

[1] Ibañez-Tallon et al., 2003, p. R27
[2] Müller et al., 2021, p. 3).
[3] Kurkowiak et al., 2015, p. 4

Figures

Figure 1 Kummer, V. (2024c). Immunofluorescence. In Biorender: medi.