

The Fight Against Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Introduction

Autosomal dominant polycystic kidney disease, ADPKD, is one of the most common, life-threatening genetic diseases. In ADPKD, fluid-filled cysts develop and enlarge in both kidneys, eventually leading to kidney failure. It is the fourth leading cause of kidney failure and more than 50 percent of people with ADPKD will develop kidney failure by age 50.

The average size of a typical kidney is a human fist. Polycystic kidneys can get much larger, some growing as large as a football, and weighing up to 30 pounds each.

Unlike some genetic diseases, ADPKD does not skip a generation meaning it often affects many people in one family. Approximately 10 percent of the people diagnosed with ADPKD have no family history of the disease, with the disease developing as a spontaneous (new) mutation. Once a person has ADPKD, even through a spontaneous mutation, they have a 50 percent chance of passing it on to each of their children.

<https://pkdcure.org/what-is-pkd/adpkd/>

Results

The process of transfection will allow exogenous DNA to be expressed by the cells and will select only the cells that express B3AR-Mcherry. Expression will be screened through the use of a fluorescent microscope which will allow to detect if cells are expressing the protein or not.

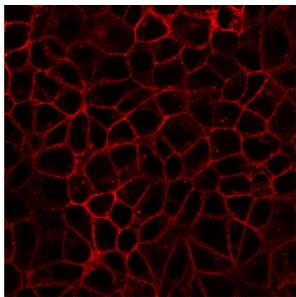


Figure 4: Null cells expressing beta-3 and m-cherry protein

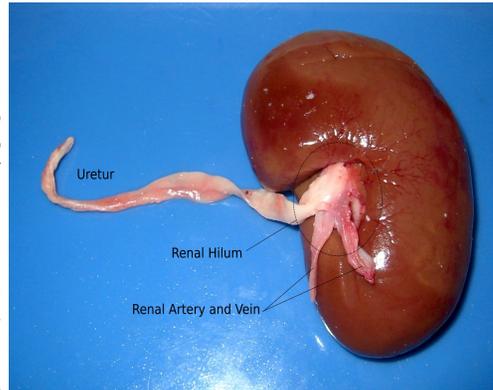


Figure 1: Healthy Human Kidney

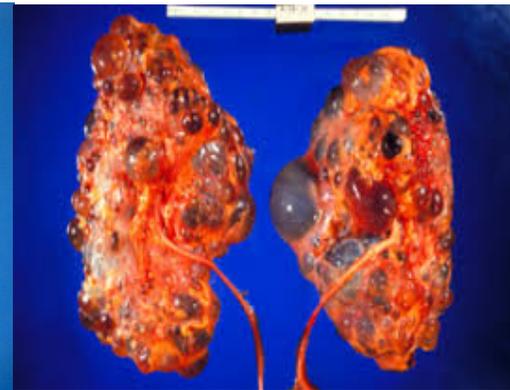


Figure 2: Human Kidney with ADPKD

Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disorder characterized by mutations in the genes that encode polycystin-1 (PC1) and polycystin-2 (PC2). Intracellular cyclic AMP (cAMP) levels are increased in ADPKD patients. Cyclic AMP (cAMP) is a second messenger involved in several cellular processes, among which cell proliferation and fluid secretion. As a consequence, cell proliferation and fluid secretion are also increased in renal cells of ADPKD patients leading to cyst formation. It is known that stimulation of vasopressin type 2 receptor (V2R), a G-protein coupled receptor, is increased in ADPKD and that in turn it causes intracellular cAMP levels to rise. The beta-3 Adrenergic Receptor (B3AR) is a G-protein coupled receptor similar to V2R in that it is expressed by kidney cells and modulates cAMP levels. The process of transfection will allow exogenous DNA to be expressed by the cells and we will select only the cells that express B3AR-Mcherry.

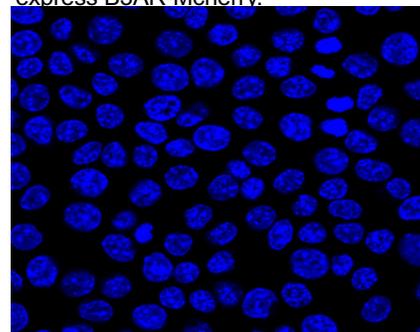


Figure 5: Nuclei of transfected null cells

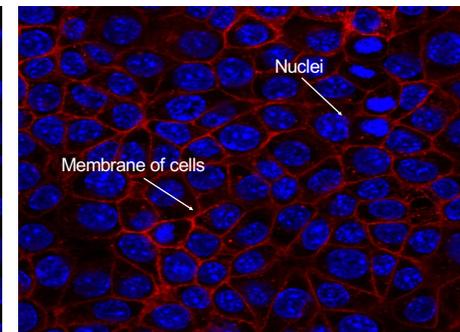


Figure 6: Transfected nulls cells expressing exogenous DNA

Methodology

Transfection is the process of inserting genetic material, such as DNA, into eukaryotic cells.

When this process takes place it will either integrate the DNA into cells or it will not. Plasmid or "circular DNA" codes for human beta-3 receptors and m-cherry. The goal is to have cells stabilized with DNA. Cells that express red membrane under fluorescent microscope gives confirmation that transfection process was successful.

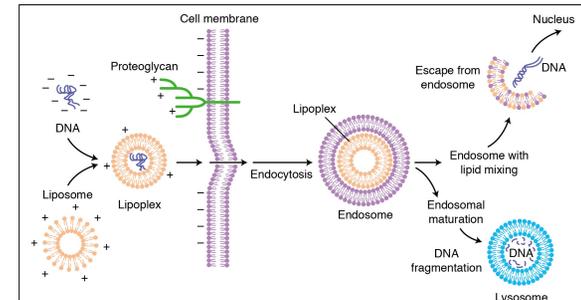


Figure 3: Process of transfection
Parker et al. 2003, Exp Rev

Conclusion

Conducting the transfection process allowed for a cell model to be generated to study the role of beta-3. Null renal epithelial cells are used to mimic human renal epithelial cells. The null cells that come from mice lack the feature of beta-3, but carry the mutation of polycystin-1. The transfection process was successful due to having beta-3 expressed in the null renal epithelial cells.